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(71) Applicant: WAYNE STATE UNIVERSITY [US/US]; 4249 Faculty Administration Bldg., 656 West Kirby, Detroit, MI 48202 (US).

(72) Inventors: GRANNEMAN, James, G. ; 5175 Shrewsbury, Troy, MI 48098 (US). LAHNERS, Kristine, N. ; 1713 Hollywood Avenue, Grosse Pointe Woods, MI 48236 (US). RAO, Donald, D. ; 605 West Madison Street #2710, Chicago, IL 60661 (US).

(74) Agent: KOHN, Kenneth, I.; Reising, Ethington, Barnard, Perry & Milton, P.O. Box 4390, Troy, MI 48099 (US).

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(54) Title: β_3-ADRENERGIC RECEPTOR PROTEIN AND DNA ENCODING SAME

(57) Abstract

Described herein is the β_3-adrenergic receptor protein and DNA which encodes the protein, vectors containing the DNA, host cells transformed with the vectors and methods of using the protein, the DNA and the transformed host cells.

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β_3-ADRENERGIC RECEPTOR PROTEIN AND DNA ENCODING SAME

GRANT REFERENCE

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FIELD OF INVENTION

The present invention relates to β_3-adrenergic receptor protein, DNA encoding the protein, the genetic elements controlling expression of the gene, and the use of host cells transformed with DNA encoding the protein for screening compounds having utility in modulating the activity of the β_3-adrenergic receptor.

BACKGROUND OF THE INVENTION

The human β_3-adrenergic receptor (β_3 receptor) gene was discovered in 1989 (L.J. Emorine et al., Sci. 245, 1989, 1118-1120). The β_3 receptor protein is widely considered to be a target for agents that will be useful as human therapeutics (J.R.S. Arch et al., Nature 309, 1984, 163-165), as well as for agents that beneficially alter the meat and fat content of

feed animals. It has been believed and repeatedly published by those that originally described the β_3 receptor gene that the rodent and human β_3 receptor genes were intronless and that the human gene contained a single exon that encoded a protein of 402 amino acids (Emorine et al., ibid; L.J. Emorine et al, Biochem. Pharmacology 41, 1991, 853-859; L.J. Emorine et al., Am. J. Clin. Nutr. 55 1992, 215S-218S; and C. Nahmias et al. EMBO Journal 10, 1991, 3721-3727). DNA constructs have been made that are based upon the assumption that the human β_3 receptor gene contains only 402 amino acids, and these constructs have demonstrated commercial value as reagents for the development of compounds that specifically interact with the β_3 receptor protein.

We have discovered that the assumption that the human β_3 receptor gene contains only one protein-coding block is incorrect. Specifically, we have discovered that the human, rat and mouse β_3 receptor genes contain two protein-coding exons. Thus, the amino acid sequence of the human and mouse β_3-adrenergic receptor proteins that were previously deduced from genomic DNA are incomplete. Most significantly, we have

discovered that the human β_3 receptor gene is 6 amino acids larger than previously believed. Because we have cloned the human receptor cDNA, we have, for the first time, elucidated the correct amino acid sequence of the human β_3 receptor.

DESCRIPTION OF THE FIGURES

Figure 1. Shows the full coding sequence for the human β_3-adrenergic receptor sequence and the deduced amino acid.

Figure 2. The structure of the full-length rat β_3 receptor gene.

Figure 3. PCR analysis of rat β_3 receptor cDNA and genomic DNA.

Figure 4. Analysis of rat adipose tissue β_3 receptor mRNA by RNase protection assay. Top: Location of cRNA probe relative to first exon/intron junction. Bottom: Autoradiogram of probe protected by white (WAT), brown (BAT) adipose tissues and liver (LIV). The CRNA probe was fully protected, indicating lack of alternative splicing in these rat tissues.

Figure 5. Comparison of the mouse and human β_3 receptor gene sequences with the homologous sequence of the first exon/intron

junction in the rat gene. Underlined are donor splice signals; the translation termination codons proposed by Emorine et al. (1989, ibid) and Nahmias et al. (1991, ibid) are in bold.

Figure 6. The nucleic acid and deduced amino acid sequences of a partial mouse β_3 receptor CDNA.

Figure 7. PCR analysis of rat and mouse genomic DNA with cDNA-derived primers. R, rat; M, mouse. See Fig. 3 for location of PCR primers.

Figure 8. Analysis of β_1 and β_3 receptor mRNA in human omental adipose tissue and in SK-N-MC cells by nuclease protection assay.

Figure 9. RNase protection analysis of human β_3 receptor mRNA expressed in SK-N-MC cells.

Figure 10. Nucleotide and amino acid sequence of a partial human β_3 receptor cDNA (p184). Fig. 10B shows the entire partial sequence and Fig. 10A shows the portion containing the second exon.

Figure 11. Analysis of β_3-receptor RNA from human white adipose tissue and SK-N-MC cells.

Figure 12. CHO cells expressing the truncated human β_3 receptor gene make mRNA encoding an unanticipated fusion protein.

Figure 13. Shows a reporter gene construct that expresses rat fat-specific elements.

Figure 14. Shows the construction of the full coding sequence for the human β_3 receptor.

SUMMARY OF THE INVENTION

The present invention provides the β_3-adrenergic receptor protein and DNA which expresses the protein. We have found as described in detail below that previous reports indicating that human β_3-adrenergic receptor protein is 402 amino acids in length are erroneous, and, in fact, the protein is 408 amino acids in length which provides the basis of the present invention.

The present invention also provides a means for transforming a host cell with a vector containing the DNA which expresses the β_3-adrenergic receptor and methods of using the transformed host cell for detecting agents, such

as chemical compounds, which affect the activity of the protein.

In another embodiment of the invention, there is provided a means for modifying the DNA which expresses the β_3-adrenergic receptor protein by site-directed mutagenesis to eliminate a donor splice site to avoid expression of fusion proteins.

Another embodiment of the invention provides oligonucleotide probes which are useful in detecting the presence of mRNA specific for the β_3-adrenergic receptor protein in cells.

The present invention further provides DNA constructs comprising fat-specific elements of mammalian DNA which expresses β_3-adrenergic receptor proteins.

Additionally, there is provided novel monoclonal antibodies to the β_3-adrenergic receptor and fragments thereof.

DETAILED DESCRIPTION OF THE INVENTION

In carrying out the work described herein, the following procedures were employed:

General recombinant DNA methods.

Standard cloning techniques used are described by Maniatis et al. (Molecular Cloning: A Laboratory

Manual, 1982, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.). RNA extraction, reverse transcription of tissue RNA and polymerase chain reaction amplification were performed as previously described by Granneman et al. (Endocrinology 130, 1992, 109-114).

Generation of β_3 cDNA probes. Probes for cloning the rat β_3 receptor cDNA and for measurement of tissue mRNA were obtained with the PCR. Brown adipose tissue (BAT) RNA (10 μg) was reverse-transcribed with a β receptor-specific (Emorine et al., 1989, ibid; Kobilka et al., Proc. Natl. Acad. Sci. 84, 1970, 46-50; and Frielle et al., Proc. Natl. Acad. Sci 84, 1987, 7920-7924) oligonucleotide, primer A, 5'-GCGAATTCGAAGGCACTICIGAAGTCGGGGCTGCGGCAGTA-3', which also contained an EcoRI restriction site on the 5' end. This cDNA was then amplified with primer A and the human β_3-specific primer 5'-GCGCTGCGCCCGGACAGCTGTGGTCCTGG-3' (Emorine et al, 1989, ibid). PCR was performed as described previously by Innis et al. (PCR Protocols, Acad. Press, San Diego, 1990, 54-59). Samples were denatured for 2 min at 94°, annealed, and extended at 72° for 4 mn. Thirty rounds of amplification were performed. One microliter of

this reaction was further amplified, described above, with the β_3-specific primer described above and a downstream primer, 5'-GCGAATTCGAA-GAAGGGCAGCCAGCAGAG-3', that is common (except for the added EcoRI site) to all β receptors (Emorine et al., 1989, ibid; Kobilka et al., 1970, ibid; and Frielle et al., 1987, ibid). The β_3 receptor PCR product was cloned into the SmaI and EcoRI sites of the plasmid pGEM 3Z (Promega) and sequenced by the dideoxynucleotide chain-termination technique (Sequenase; United States Biochemical Corp). The PCR product was found to be highly homologous to the human β_3 receptor gene (Emorine et al., 1989, ibid) and, ultimately, identical to a rat cDNA clone encoding the rat β receptor.

Library construction and screening (rat).

Library construction, screening, and cloning were performed using standard techniques (Maniatis et al., 1982, ibid). A cDNA library was constructed in LambdaGEM-4 (Promega) using poly(A)$^+$ RNA isolated from BAT of cold-exposed rats. This library contained approximately 3 x 10^6 recombinants, with an average insert size of 1.5 kb. Three hundred thousand recombinants were screened at high stringency (0.03 M NaCl, 3 mM

sodium citrate, pH 7, at 55°) with the cloned rat β_3 PCR product labeled with (^{32}P)dCTP using random primers (Maniatis et al., ibid). Twenty-seven phage were isolated from the amplified library, and two plasmids (p108 and p109) of the same size (about 1.73 kb) were rescued. Sequencing of p108 and p109 from the 5' ends indicated they were identical and truncated with respect to the predicted initiation codon of the human β_3 receptor sequence (Emorine et al., 1989, ibid). Screening of the remaining isolates by PCR failed to detect any full-length cDNAs, and primer extension experiments with tissue mRNA suggested that secondary structure, owing to high G-C content, may have limited the ability of the reverse transcriptase to synthesize cDNA through the missing 5' region. Therefore, to obtain the remaining sequence, a Sprague-Dawley rat genomic library (Clontech) was screened with a p108 probe to obtain the rat genomic sequence. The rat β_3 gene was identified by sequencing four hundred forty-four base pairs of genomic sequence that overlapped with p108 β_3 receptor DNA. A full-length clone was then produced by cloning the genomic sequence from bases -104 to +390 (relative to translation initiation) into the

AccI site of p108. Both DNA strands were sequenced by the dideoxy chain-termination technique (Maniatis et al., ibid), and no discrepancies were found.

Transfection of CHO-k1 cells. The assembled β_3 receptor construct was cloned into pRC/CMV (Invitrogen), an expression vector containing the cytomegalovirus promoter and a neomycin resistance gene. This construct was transfected into CHO-k1 cells using the $CaPO_4$ method. Stably transfected cells were selected in the presence of Geneticin (800 μg/ml) and pooled for further analysis.

Numerous eucaryotic cells can be used. Preferably, these cells will not express any related β adrenergic receptor (i.e., β_1, β_2, or β_3 receptors). Examples of such cells include Chinese hamster ovary cells, murine B-82 cells, murine adrenal cortical Y1 cells, xenopus oocytes, or insect Sf cells.

Numerous vectors, some with promoters that are geared to specific cell types can be used. Examples are inducible promoters like mouse mammary tumor virus (MMTV) promoter or metalothionin promoter. Others include retrovirus vectors for gene therapy. Based upon

the information in Example 1 below, numerous variations are possible.

Adenylyl cyclase assay. Adenylyl cyclase activity was determined by the method of Salomon (Adv. Cyclic Nucleotide Res. 10, 1979, 35-55). Culture medium was removed and cells were washed in phosphate-buffered saline and then harvested in 25 mM HEPES (pH 8.0) buffer containing 2 mM $MgCl_2$ and 1 mM EDTA. Cell were homogenized and centrifuged at 48,000 x g for 15 min. to obtain crude membranes. Membrane pellets were resuspended and used directly or frozen at -80° until used. Freezing did not affect activity. Membranes (5-15 μg of protein) were preincubated at 4°, in a volume of 40 μl, with the specified drugs for 15 min. Adenylyl cyclase reactions were initiated by addition of substrate mixture and were terminated after 30 min at 30°. BAT membrane adenylyl cyclase activity was determined as previously described (Granneman et al., J. Pharmacol. Exp. Ther. 254, 1990, 508-513, and Granneman et al., J. Pharmacol. Exp. Ther. 256, 1991, 412-425), using membranes from 7-day-old rats. Concentration-response data were analyzed by nonlinear regression analysis with a one-site mass action equation for transfected CHO

cells (Enzfitter, Elsevier Biosoft). A two-site model was used to analyze catecholamine-stimulated adenylyl cyclase in BAT, with the low affinity component representing stimulation by β_3 receptors (Chaudhry et al., Am. Jour. Physiol. 261, 1991, R403-R411).

Tissue mRNA analysis. The size of the β_3 receptor transcripts was determined by Northern blot analysis of rat poly(A)$^+$ RNA, as previously described (Maniatis et al., ibid; and Granneman et al., Endocrinology 125, 1989, 2328-2335). The cDNA probe used corresponded to bp 228-665 of Fig. 1 and was labeled by random primers. Tissue mRNA distribution experiments were conducted on total RNA with a solution hybridization assay (Maniatis et al., ibid; and Granneman et al., Endocrinology 127, 1990, 1596-1601). The radioactive cRNA probe used was transcribed in vitro from the cloned β_3 receptor PCR product (p110) with [^{32}P]CTP, using the T7 promoter. The probe corresponded to bp 746-917 in Fig. 13. Tissue or cellular RNA (6-50 µg) was co-precipitated with 3 x 10^4 cpm of the ^{32}P-labeled cRNA probe. Samples were hybridized at 55° for 12-18 hr and then diluted, and the nonhybridized probe was digested with 300 units

of T-1 ribonuclease for 45 min at 37°. The [^{32}P]RNA probe that was protected from RNase digestion was electrophoretically resolved on a denaturing polyacrylamide gel containing 8 M urea. The gels were dried and exposed to Kodak XAR-5 film for 18-72 hr.

Analysis of β_3 receptor mRNA by RNase protection assay. Rat and human β_3 receptor mRNAs were analyzed by RNase protection assay using species-specific probes. The rat probe used (p152) was the BssHII to BglII fragment of the cloned rat β_3 cDNA cloned into pGEM-7z. This sequence spans the first exon/intron junction.

Human mRNA was mapped with a β_1 receptor probe and two β_3 receptor probes that were amplified from human genomic DNA. A β_3 receptor (p146) and the β_1 (p145) probes were amplified by "nested" PCR (Granneman et al. Molecular Pharmacol. 40, 1991, 895-899) from total nucleic acids using primers based upon the published sequences (Emorine et al., 1989, ibid and Frielle et al., 1987, ibid). The resulting receptor DNAs were shortened and cloned into pGEM-7z for the generation of riboprobes. These probes are exact matches of the published sequences and encode amino acids 178-271 (β_1) and

151 to 223 (β_3). The second human β_3 receptor probe was amplified from genomic DNA (Promega) with a primer set that was designed to amplify a 256 bp DNA fragment which spanned the putative donor splice site. The coding strand primer (HB3G+) was 5'TGCGAATTCTGCCTTCAACCCGCTC 3' and the noncoding strand primer was 5' GCAGGATCCACGGACACATCGCATGCTTCC 3'. Both primers were based upon the published human sequence and contained engineered restriction sites of the 5' ends for cloning into pGEM-7z (p174). The sequence of p174 was an exact match of the published human β_3 receptor gene sequence except for a discrepancy of A for G in the published sequence at bp at 1193 (5, GenBank accession #M29932). This potential discrepancy does not affect the nuclease protection assay because the T-1 ribonuclease used does not cleave at A (J. N. Davidson, The Biochem. of Nucleic Acids, 7th ed., 1972, Academic Press, New York), and no fragments indicative of cleavage at this site were detected.

Cloning of a partial mouse β_3 receptor cDNA. The mouse β_3 receptor cDNA was obtained from mouse white adipose tissue RNA by reverse

transcription/PCR (Granneman et al., 1992, ibid). Reverse transcription of total RNA was performed with the oligonucleotide primer 5' ATTAAAAGGTTTGCATC 3' that was based upon the rat cDNA (Granneman et al., 1991, ibid). The resulting cDNA was then amplified by PCR. The coding strand primer was 5' GGACTTTCGCGACGCCT 3' and the noncoding strand primer was 5' GCATCCATGGACGTTGCTTGTC 3', which were also based upon the rat sequence. Samples were denatured at 94°C for 2 min., annealed at 63°C for 1.5 min and extended at 72°C for 2 min for 30 cycles. The resulting PCR product was shortened to 180 bp, cloned into pGEM-7z (p158) and sequenced.

PCR analysis of mouse and rat genomic DNA. To estimate the size of the mouse intron(s), PCR analysis was conducted on mouse and rat genomic DNA. The primer set used was the same that was used above to amplify the mouse cDNA. PCR was carried out for 30 cycles using 1 μg of mouse or rat genomic DNA (Promega) as described above. PCR products were resolved on 1% agarose gel containing ethidium bromide and visualized with ultraviolet light. The identity of these products was verified by Southern blot analysis with an internal probe from the rat cDNA.

Cell culture. SK-N-MC cells were grown in Dulbecco's modified Eagle's medium containing 10% fetal calf serum (Hyclone), penicillin (100,000 units/1) and streptomycin (100 mg/1). Cells were subcultured at a ratio of 1:10 and harvested when about 80% confluent.

Mammalian tissues. Rat tissues were obtained from male Sprague-Dawley rats and mouse tissues were from male outbred mice (Hilltop Labs). Human adipose tissue was obtained with informed consent from surgical specimens.

The discovery of the authentic amino acid sequence of the human β-adrenergic receptor represents a significant improvement in the state of the art with respect to technologies surrounding the β_3 receptor. Specifically, cells expressing the correct amino acid sequence will be most preferable to screen agents for human and animal use. The full length (408 amino acids) human β_3-adrenergic receptor we have discovered has a pharmacological profile that is different from the truncated (402 amino acids) receptor previously reported. Pindolol derivatives and BRL3744 are partial agonists at both the full length and truncated receptors. However, there is a dramatic difference in the differential

potency of typical β-adrenergic receptor antagonists. Propranolol and alprenolol inhibit the full length receptor with submicromolar potencies, but have been reported to be essentially inactive at the truncated receptor (Emorine et al., 1989, ibid). It is difficult to compare the potencies of agonists because of possible differences in receptor reserves, and problems in comparing data from binding and functional measurements. Based on the limited data available, however, it appears that CYP is about 100-fold less potent at the full length receptor than at the truncated receptor. Pindolol has been reported to have EC_{50} values of 150 nM or 1100 nM in truncated β_3-transfected CHO cells, while we found an EC_{50} of 2800 nM, although a K_I of 84 nM in these cells. Similarly, the EC_{50} for BRL 37344 in truncated β_3-transfected CHO cells has been reported to be 6 nM and 180 nM, but we found it to be 840 nM in cells which produce the full-length receptor. It appears that the full-length receptor more closely resembles the "atypical" β-adrenergic receptor found in cardiac, intestinal and adipose tissues and the cloned rat β_3-receptor than the cloned human truncated β_3-receptor. Similarities

include 1) a submicromolar affinity for classical β-adrenergic receptor antagonists; 2) a midnanomolar affinity for CYP; 3) activation by pindolol derivatives with low potency; and 4) a relatively low potency of BRL 37344.

The rodent β_3 receptor is abundantly expressed only in adipose tissue (Granneman et al., 1991, ibid). In this regard, the original tissue distribution of the β_3 receptor mRNA described by Emorine et al. (1989, ibid) was erroneous because most of the probe that was used was derived from the first intron and the exon sequence used had no homology with the rat tissues tested. These observations indicate that the β_3 receptor gene contains elements involved in adipose tissue-specific expression. We have isolated the rat β_3 receptor gene, and have identified genetic elements that are likely to be involved in this phenotypic expression. Tissue-specific enhancers have been identified in the first intron of several genes (Brooks et al., J. Biol. Chem. 266, 1991, 7848-7859, and Parmacek et al., J. Biol. Chem. 265, 1990, 15970-15976). We have found the sequence within and surrounding one of the inverted repeats in the first intron of the β_3 receptor gene bears striking homology

with NF-1 (Santaro et al., Natur. 334, 1988, 218-224 and with ARF6 (Graves et al., Mol. Cell. Biol. 12, 1992, 1202-1208). It is anticipated that these sequences are involved in the adipose tissue-specific expression of the β_3 receptor based on recent reports that sequences related to NF-1 and ARF6 are involved in the control of adipose tissue-specific gene expression (Graves et al., 1992, ibid. and Genes Dev. 5, 1991, 428-437). The modulation of tissue-specific genes represents a new approach in the treatment of certain diseases and in the generation of agents that produce desirable characteristics in meat-producing animals. For example, agents like Cigilazone that are being developed as antidiabetes therapeutics augment the expression of adipose tissue-specific genes (Kletzien et al., Mol. Pharmacol 41, 1992, 393-398). Efforts to identify novel agents that modify fat-specific gene expression will be facilitated greatly by cell lines expressing readily-detected reporter genes whose transcription is governed by adipose tissue-specific promoter elements. Promoter/reporter gene constructs that are based upon the fat-specific elements within the β_3 receptor gene

represent a novel, useful approach for developing gene-modulating agents.

The structure of DNA sequence of the gene encoding the human β_3-adrenergic receptor and the amino acid sequence of the receptor was reported by Emorine et al. (1989, ibid). The receptor was identified by Emorine et al. as having 402 amino acids, which we have now found to be erroneous, encoded from a single exon. As noted above, we have discovered that the human β_3-adrenergic gene has two coding exons, and the amino acid sequence for the protein is 408 amino acids in length. We have found that the TAG codon believed to be a termination codon is in a position to contain a human donor splice site (GT) as is more fully detailed below.

The discovery of the donor splice signal in the β_3-adrenergic receptor gene was initially found in the rat gene. A rat genomic library was screened with the rat β_3 receptor cDNA and isolated a clone containing a 12.1 kb insert. This clone was then subjected to Southern blot analysis using the rat β_3 cDNA as a probe. Digestion of the genomic clone with Xho I revealed prominent bands of 3, 4 and .6 kb that hybridized to the rat β_3 receptor cDNA. Because

the rat β_3 receptor cDNA contains only a single Xho I site, these data suggested the existence of one or more introns in the rat β_3 receptor. Further analysis utilizing selective cDNA probes suggested the existence of intron(s) near the 3' end of the coding region. The Xho I fragments derived from the genomic clone were then isolated and sequenced.

Shown in figure 2A is a restriction map of the rat β_3 receptor gene and the exon/intron structure of the rat β_3 receptor gene that was deduced by comparison of the genomic sequence with the cDNA (Fig. 2B). "A" shows a map of the rat β_3 receptor gene illustrating the locations of restriction enzyme cleavage sites and the translation initiation (ATG) and termination (TGA) codons. Sequences within this map are contained in the plasmids p111, p108 and p167. "B" shows a schematic representation of the rat β_3 receptor gene, with mature mRNA blocked and the coding sequence filled. E, exon; I, intron. "C" shows a nucleic acid and amino acid sequences of exon/intron junctions of the rat β_3 receptor gene, beginning with Pro^{374}. Underlined are the donor and acceptor splice sites. The inverted repeat that has homology with NF-1 is in bold.

The rat β_3 receptor gene contains three exons and two introns. The first intron interrupts the open reading frame 12 amino acids from the carboxyl end (Fig. 2C). This intron is 394 bp and contains both 5' donor and 3' acceptor splicing signals. The second exon is 68 bp long and encodes the translation termination codon and 28 bp of nontranslated sequence. The second intron is 207 bp long and also contains donor and acceptor splice signals. The final exon contains sequences through the polyadenylation signal as described by Granneman et al. (Molecular Phar. 40, 1991, 895-899).

To further verify that the rat β_3 receptor gene contains introns, we performed PCR analysis of rat β_3 receptor cDNA (prepared by reverse transcription of total RNA from adipose tissue) and genomic DNA. PCR primers were complementary to sequences in the first and third exons (Fig. 3A). The coding strand primer was placed upstream of the first splice junction, whereas the noncoding primer was placed in the third exon. Thus, the expected PCR product spanned the introns. As expected, this primer set amplified a 845 bp fragment from genomic DNA, as shown in lane 2 of Fig. 3. When tissue cDNA

was used as a template, the product was 246 bp (see lane 1 of Fig 3), as was expected if the primary transcript contained introns which had been removed. No other PCR products were observed, indicating that the β_3 pre-mRNA is not alternatively-placed. To further verify this conclusion, nuclease protection assay was performed on rat β_3 receptor mRNA. The probe used (p152) in this instance was derived from the cloned rat β_3 receptor cDNA and spanned the first exon/intron junction (Fig. 4). If both introns of the rat β_3 receptor are removed by RNA splicing, then tissue mRNA should protect the full (281 nt) complementary probe. However, if the first donor site is not used (i.e., is alternatively spliced), then a fragment of 232 nucleotides would be protected by tissue β_3 receptor mRNA. As shown in Fig. 4, RNA from both white (WAT) and brown (BAT) adipose tissues protected the full probe and no smaller fragments indicative of alternative splicing were observed. As expected, RNA from liver (LIV) failed to protect the β_3 receptor probe indicating that the expression of the gene is adipose tissue-specific (see also Granneman et al., Endocrinology 130, 1992, 109-114).

The murine and, as noted above, human β_3-adrenergic receptor genes have been cloned recently, and both were assumed to lack introns (Emorine et al., 1989, ibid, and Nahmias et al., 1991, ibid). However, analysis of the genomic sequence alone is not sufficient to decide whether this is so. As shown in Fig. 5, the first exon/intron junction of the rat gene contains the sequence AGGTAG. In the absence of information derived from cDNA, it might be concluded erroneously that the final amino acid is arginine (encoded by AGG) followed by a translation termination codon (TAG). In this regard, we noticed the sequence of the mouse β_3 receptor gene is identical to that of the rat in this region (Nahmias et al., ibid). In addition, the human gene also contains the sequence GGTAG in a homologous site, and this sequence has been found to contain a donor splice site (GT), in which case the coding sequence continues, or it could be a termination codon (TAG), as originally deduced.

In order to verify that the mouse gene contains introns, we cloned the relevant region from mouse adipose tissue by reverse transcription of RNA followed by PCR. The

nucleic acid sequence and deduced amino acid sequence of the mouse β_3 receptor cDNA is shown in Fig. 6. The partial cDNA was cloned by reverse transcription of mouse adipose tissue mRNA followed by PCR. Shown is sequence beginning with the codon for Val^{378}. The cDNA exactly matches the genomic sequence reported by Nahmias et al. (1991, ibid) until Arg^{388}. The open reading frame continues for 12 more amino acids (Bold, Phe Asp Gly Tyr Glu Gly Ala Arg Pro Phe Pro Thr), which are identical to the rat sequence. The 45 bases of nontranslated sequence in this clone are 71% identical to the non-translated sequence of the rat β_3 receptor cDNA.

To further verify that the mouse β_3 receptor gene contains introns and to estimate their size, PCR analysis of genomic DNA was performed with oligonucleotide primers that were based upon cDNA and were designed to span the intron(s). In the mouse cDNA, there are 208 bp between the primers in this set. Amplification of genomic DNA with this primer set resulted in a PCR product that was about 985 bp, confirming that the mouse gene contains introns and further indicating that the intron(s) present in the

mouse gene are about 120 bp larger than those in the rat gene (Fig. 7).

In order to determine whether the human gene contains introns, we first identified a source of human β_3 receptor mRNA for comparison. In rats, the β_3 adrenergic receptor is expressed abundantly only in adipose tissue, where β_3 receptor mRNA is about 5-7 times more abundant than β_1 receptor mRNA (Granneman et al., Endocrinology 130, 1992, 109-114). We examined mRNA from human subcutaneous and omental adipose tissues by RNAse protection assay, and although β_1 receptor mRNA could be readily detected by nuclease protection assay, transcripts encoding the β_3 receptor were absent at the detection limit of the assay (about 4 copies per cell) (Fig. 8). 50μg of total RNA was hybridized to human β_1 (p145) and β_3 (p146) receptor probes simultaneously. SK-N-MC cells contain both β_1 and β_3 receptor mRNA, while human omental adipose tissue contains only β_1 receptor transcripts. Right lane shows synthetic human β_3 receptor RNA standards. Thus, although the β_3 receptor does not appear to be expressed in human subcutaneous or omental adipose tissue, we did find that the β_3 receptor is abundantly expressed along with

the β_1 receptor in the human neuroblastoma cell line SK-N-MC. Thus, these cells provide an excellent source for analysis of the human β_3 mRNA (Fig. 8).

We mapped the 3' end of the β_3-adrenergic receptor mRNA from SK-N-MC cells. The probe we used (p174) was derived from human genomic DNA and was designed to span the putative translation termination site/donor splice site (Fig 9, A; see also Fig 5). Referring to Fig. 9, the cRNA probe derived from p146 is complementary to sequence within the first exon of the human β_3 receptor and is fully protected by SK-N-MC mRNA. The cRNA probe derived from p174 is complementary to genomic DNA sequence that spans the putative first exon/intron junction (Fig. 9A). Although SK-N-MC β_3 mRNA protects the full p174 cRNA probe (256 nt), most β_3 transcripts utilize the donor splice signal as indicated by the protected fragment of 194 nt. If the β_3 receptor gene is intronless, then SK-N-MC RNA should fully protect the complementary 256 nucleotide probe. However, if the 5' donor splicing signal contained in the human β_3 receptor pre-mRNA is utilized in the SK-N-MC cells, then cellular RNA should protect exactly 194 nucleotides of the probe. We found

that both 256 and 194 nt of the probe was protected by SK-N-MC RNA (Fig. 9). The ability of SK-N-MC RNA to protect 194 nt of the probe indicated that the splice signals in the human β_3 receptor primary transcript are used by SK-N-MC cells, and thus, the gene contains at least one intron. However, unlike the expression of the rat β_3 receptor gene in adipocytes, the efficiency of splicing was not complete, as indicated by the 256 nt fragment. Thus, about one-fourth of the total β_3 receptor mRNA failed to undergo splicing; and, as originally proposed (Emorine et al., 1989, ibid), the translation of the protein would be predicted to terminate at this point. Nevertheless, the great majority of the transcripts were spliced by these cells, and it seemed likely that the human β_3 receptor gene encoded additional amino acids.

To verify whether the spliced human β_3 mRNA encodes additional amino acids, the relevant region of the human β_3 receptor cDNA from SK-N-MC cells was cloned using RACE. Shown in Fig. 10 are the nucleic acid and deduced amino acid sequences of the human β_3 receptor cDNA we obtained. Shown in 10B is the human β_3 receptor cDNA (p184) that was obtained from SK-N-MC cells

using RACE, beginning with the codon for Ala^{392} (Fig. 10A). The 5' cDNA sequence of the clone is identical to the published sequence of the human gene (Emorine et al., 1991, ibid) for 194 bp, then diverges (Bold) exactly at the predicted 5' donor site. The open reading frame continued for 6 amino acids, followed by 657 bp of nontranslated sequence. Fig. 10B shows the complete nucleic acid sequence of p184. Shown in bold is sequence encoding the novel exon(s). Example 1 sets forth further details of the cloning of p184.

We also verified that the cDNA sequence obtained from SK-N-MC cells was in fact expressed in normal human tissues (Fig. 11). Normal human adipose tissue expresses β_3 receptor mRNA containing two protein-coding exons. Details of this experiment are set forth in Example 5 hereof.

To Further verify the GT donor splice site, RNA was obtained from CHO cells that had been transfected to express the truncated (encoding 402 amino acids) human β_3 receptor gene and was subjected to RNase protection analysis with a cRNA probe derived from the human β_3 receptor gene (p174, Fig. 9). CHO cellular RNA

protected both 256 nt and 194 nt of the cRNA probe. The presence of the 194 nt fragment demonstrates that the 5' donor splice signal present in the gene is utilized by CHO cells, and results in the splicing of the first exon with sequences with the expression vector or at the site of DNA integration. Such splicing would be expected to produce a fusion protein, making cells that express such constructs unacceptable for drug screening.

Example 3 set forth below describes means for eliminating fusion proteins by site-directed mutagenesis. The purpose of the site-directed mutagenesis is to alter the codon for gly^{402} so as to eliminate the donor splice signal in order to prevent production of fusion proteins. This modification is important because the splicing of the β_3 receptor premRNA is not complete and can potentially encode both a 402 amino acid receptor, as well as a fusion protein.

EXAMPLE 1

Cloning of a partial human β_3 receptor cDNA (p184).

A partial human β_3 receptor cDNA was cloned by the rapid amplification of cDNA ends (RACE) technique (Frohman et al., 1990, ibid). General cloning techniques used are described in Maniatis et al., ibid. Total RNA (10 μg) from SK-N-MC cells was reverse-transcribed as described previously (Granneman, et al., 1991, Molecular Pharmacol. 40, 895-899) with a 17mer poly T deoxyoligonucleotide primer containing an engineered XbaI an BamHI restriction sites on the 5' end (5' ACTATAGGGTCTAGAGGATCCGTTTTTTTTTT-TTTTTTT 3'). The resulting cDNA was amplified with the human β_3 coding strand was 5' TGCGAATTCTGCCTTCAACCCGCTC 3. The noncoding strand primer was 5' ACTATAGGGTCTAGAGGATCCG 3', which was the adapter sequence of the primer/adapter oligonucleotide described above. PCR was performed for 30 rounds as follows: Samples were denatured at 94°C for 2 min., annealed at 58°C for 2 min. and extended at 72°C for 4 min. The resulting products digested with EcoRI and XbaI, then cloned into pGEM-7z. Twelve recombinants were screened to determine insert size. Analysis of two clones by RNase protection

assay with the p174 cRNA probe indicated that the 870 bp inserts they contained encoded a human β_3 receptor cDNA. These clones were then analyzed by restriction mapping and dideoxynucleotide sequencing, and were found to be the same. The complete nucleotide sequence of p184 is given in Fig. 10.

EXAMPLE 2

Gene construct Encoding Full-Length (408 a.a.) Human β_3-Adrenergic Receptor.

(A) DNA

Such constructs are made as follows: A human β_3 receptor genomic clone is obtained by screening a human genomic library (Clontech) with a radiolabelled probe derived from p184, described above in Example 1. The phage DNA is digested with BglII and BamHI and this 2 kb fragment cloned into pGEM-7z. This construct contains the first exon and part of the first intron of the human β_3 receptor gene. This construct is digested with TaqI (in exon 1) and XbaI (in the vector polylinker). The TaqI to XbaI fragment is removed and the two fragments, containing sequence from XbaI to TaqI (vector/5' gene) and sequence from TaqI to TaqI (in exon 1), is recovered. A three-way ligation is performed

using the fragments just described and the TaqI to XbaI fragment of p184. The resulting construct contains an open reading frame that encodes the full-length human β_3 receptor. This construct and its preparation are depicted schematically in Fig. 14.

Alternatively, DNA encoding the full-length human β_3 receptor is also obtained by oligonucleotide-directed mutagenesis of truncated (402 amino acid) clones, using commercially-available kits (e.g. Amersham) DNA sequence encoding exon 1 and more than 25 bp of the first intron of the human beta 3 gene (Emorine et al., 1989, ibid) is cloned into a M-13 vector, or equivalent, single-stranded vector. Either the coding strand (Fig. 1) or complementary strand may be used. The single-stranded DNA just described is hybridized to an oligonucleotide containing sequence that is complementary to the native genomic strand. The 5' end of the oligonucleotide is complementary to the end of exon 1 to the splice site (Fig. 10A). The next 19 nucleotides begin with G, followed by the codons for ala ser trp gly val ser. The 3' end of the oligonucleotide continues with the sequence at the start of the first intron. For

example: the oligonucleotide 5' CCAGGCTTTGCC-AACGGCTCGACGGGGCTTCTTGGGGAGTTTCTTAGGTAACCGGGGCA-GAGGGACC 3' (or its complement) is hybridized to the appropriate single stranded DNA. Useful variants of the oligonucleotide include those that are somewhat longer or shorter on the 5' or 3' ends. The oligonucleotide is extended with Klenow polymerase using dCTPαS, and ligated. Single-stranded DNA is removed, and the native strand is then nicked with NciI and digested with exonuclease III. The DNA is repolymerized and ligated, then transformed into host cell (e.g. E. coli).

(B) Procaryotic and eucaryotic vectors containing DNA described in (A)

The DNA sequence encoding the 408 amino acid human β_3 receptor protein is first cloned into an appropriate commercially available vector for propagation of bacteria. In the present example, the insert described above is cloned into pGEM-7z. The protein-coding insert is then shuttled into appropriate mammalian expression vectors. In this case, we use pRc/CMV (Invitrogen), an expression vector containing the cytomegalovirus promoter and neomycin resistance gene. In the case of pRc/CMV, we take advantage

of the HindIII and XbaI sites in the vector to shuttle the insert from pGEM-7z.

(C) Host cells that have been transfected to express the proteins encoded by the DNA constructs described in (B).

(1) Procaryotic cells used to propagate the plasmids are various strains of E. coli, including JM109, HB101 and DH5α. These cells are transformed using standard techniques known to the art.

(2) Eucaryotic cells

Chinese hamster ovary (CHO) cells are transfected with constructs based upon the expression vector pRc/CMV. CHO cells are preferred because they do not natively express any known β receptor subtype. Transfection of CHO cells with DNA constructs is accomplished by the $CaPO_4$-DNA precipitation method as described in Maniatis et al., ibid. To obtain cells that stably express these DNA constructs, transfected cells are selected based upon their resistance to G418, which is conferred by the neomycin-resistance gene contained in pRc/CMV. Cells that survive selective conditions (e.g. 800 μg/ml G418) are then cloned by limiting dilution. The stable expression of the human β_3 receptor is

verified by (a) the presence of human β_3, receptor mRNA, determined by nuclease protection assay (see below), and (b) by the stimulation of adenylyl cyclase (e.g., Granneman et al. ibid) with selective β_3 receptor agonists such as BRL 37344 (1 μM), as well as the stimulation by isoproterenol (10 μM) that is resistant to blockade by CGP 20712A (100 nM).

EXAMPLE 3

Construct encoding alternative (402a.a.) human β_3 receptor wherein Gly^{402} is degenerate.

DNA constructs encoding the first exon of the human β_3 receptor in which the codon for $glycine^{402}$ is made degenerate to alter the sequence GGGTAG so as to eliminate the donor splice signal is prepared by site-directed mutagenesis is performed using a commercially available kit (Amersham) as described in Example 2, except that the oligonucleotide is 5' CCAGGCTTTGCCAACGGCTCGACGG(T/C/A)TAGGTAACCGGGGCAGA GGGACC 3'. Following this procedure the sequence GGGTAG will be changed to GGTTAG, GGCTAG, and GGATAG, respectively.

EXAMPLE 4

A method for using the cells described in Example 2C to screen agents that specifically interact (either as agonists or antagonists) with the protein product of such DNA sequences.

CHO cells expressing the full length human β_3 receptor are harvested and membranes prepared as described by Granneman et al., ibid. Adenylyl cyclase activity is then determined in response to various agents known or thought to interact with the β_3 receptor, using the method of Salomon, ibid. Agonists are identified by the ability to increase cyclic AMP generation above basal levels. Antagonists are identified by their ability to decrease adenylyl cyclase activity that is stimulated by 100 nM isoproterenol.

The β_3 receptor is known to increase the formation of cyclic AMP; thus, the interaction of compounds with the recombinant proteins are monitored by changes in cyclic AMP (in whole cells or in cell membranes), or by monitoring the consequences of cyclic AMP formation. There are numerous ways to monitor cyclic AMP, including RIA and fluorescence immunoassay. In addition, the β_3 receptor may activate non-cyclic AMP responses, e.g. calcium

influx. Therefore, there are several functional responses that are the consequence of the activation of these receptors.

Most preferably, these cells will be used to screen compounds that have potential antiobesity, antidiabetes and antispasmodic actions. These cells may also be of benefit in the screening of agents that alter body composition (e.g. repartitioning agents) of meat-producing animals.

EXAMPLE 5

p192 and a method of its use in the detection of human β_3 mRNA.

p184 (see Figs. 10 and 14) contains sequences that are useful in the analysis and detection of mRNA encoding the human β_3 receptor. to obtain one such sequence (p192) the EcoRI to NcoI fragment of p184 was cloned into pGEM-7z. The insert of p192 contains the first 292 bp of p184 and spans exon 1 and exon 2. This construct is used to generate cRNA probes for specific detection of human β_3 receptor mRNA or cDNA, using standard techniques. Shown in Fig. 11 is the use of p192 to detect human β_3 receptor cDNA that had been amplified with PCR. This was performed as follows: RNA from human adipose

tissue and SK-N-MC cells was reverse transcribed with an oligonucleotide primer (5'CAACAGAG-TTGTTGCTTCTTGTCC 3') that was based upon exon 2 of the cDNA derived from SK-N-MC cells. The resulting cDNA amplified by PCR with this primer and primer HB3G+ (see methods). PCR products were then identified by nuclease protection assay with gene- (p174 - Fig 9) and cDNA-derived (p192) probes (Fig. 11). The fact that human adipose tissue cDNA protects exactly 247 nt of the p192 probe and 194 nt of the p174 probe demonstrate that mRNA corresponding to the novel β_3 receptor cDNA we have cloned from SK-N-MC cells is expressed in normal human adipose tissue (see Fig. 11).

A number of sequences in p184 are useful for diagnosis. These include the PCR primers described above. In general, all sequences that hybridize to either strand of p184 are useful. Most preferably, these are sequences (like p192) that can be used to distinguish β_3 receptor mRNA from genomic DNA by DNA amplification techniques (e.g. polymerase chain reaction, for example see Fig. 11) or that can be used to identify or quantify human β_3 receptor mRNA or mRNA splice variants (e.g. ribonuclease

protection assay or RNA hybridization blot analysis (for example Fig. 12). Such sequences are useful in monitoring β_3 receptor gene expression for diagnosis or for development of agents that alter β_3 receptor expression.

EXAMPLE 6

Antibodies that are directed against the amino acid sequence: alanine serine tryptophan glycine valine serine.

Polyclonal and monoclonal antibodies are generated against the synthetic peptide by conventional techniques using commercially available services (Chiron, Emeryville, CA). To determine levels of expression of the β_3 receptor, antibodies may be useful in diagnosis to determine levels of expression of the β_3 receptor.

EXAMPLE 7

DNA constructs containing sequences within the introns or 5' flanking regions of the rat β_3 receptor gene (see Fig. 2).

The rat β_3 receptor gene is expressed in a fat-specific fashion (Granneman et al., Molecular Pharmacol., 1991, 40, 895-899). Thus, the gene contains elements that confer fat-specific expression. To obtain the rat β_3

receptor gene, including the elements that control its expression in fat cells, we screened a rat cDNA library with a rat β_3 cDNA clone. DNA fragments of the gene and cDNA were cloned so as to obtain the DNA sequence of the entire rat gene. The location of the clones obtained are given in Fig. 2 p111 is a 3 kb XhoI to SmaI fragment containing the rat β_3 receptor 5' flanking promoter region); p108 contains the internal 211 bp SmaI to XhoI fragment and p167 contains the 2.6 kb XhoI to SphI fragment, which includes the first and second introns and the second and third exons. Sequence analysis of the first intron of the rat β_3 receptor gene indicates it contains elements involved in fat-specific gene expression.

EXAMPLE 8

Reporter gene constructs that contain elements described in Example 7, that are designed to modify the cellular transcription of the reporter gene.

The following construct (p182) has been made: The NheI to EcoRV fragment of p167, containing the first and second introns of the rat β_3 receptor gene (see 7 above), was cloned into pCAT promoter vector (Promega, Madison WI).

This construct contains the SV40 promoter, and the introns have been cloned in such a way as to modify the activity of the promoter (Fig. 13). Although we used the chloramphenicol acetyltransferase reporter, there are numerous reporter genes that can be used, e.g. beta galactosidase, luciferase, inter alia). Alternatively, fat-specific elements, especially those in the 5'flanking region contained in p111 could be used in the construct.

EXAMPLE 9

Mammalian cells expressing p182.

3T3-F442A cells are stably transfected with constructs described in Example 8 by co-transfecting with pRC/CMV and selection with G418. Stable tranformants are identified by increase in reporter gene activity with insulin, and a decrease in reporter gene activity with tumor necrosis factor α.

Other appropriate cells into which the construct could be transfected include those that express fat-specific transcription factors or demonstrate the ability to differentiate into an adipocyte phenotype in vitro. Examples of such cells are 3T3-F442A cells, 3T3-L1 cells and RMT

preadipose cells. Included are the nondifferentiated phenotypes of these cells.

Either transient or stable transfections can be used. These can be accomplished by numerous techniques, including $CaPO_4$, and liposome-mediated transfer (Transfectam, Promega, Madison, Wisconsin) and electroporation. The activity of the reporter gene is monitored by commercially-available kits (e.g. Promega, Madison, Wisconsin).

EXAMPLE 10

A method for using cells described in Example 9 to screen agents for gene-modulating activity.

3T3-F442A cells that have been stably transfected with a β_3 promoter/β-galactosidase reporter gene construct (p182) are plated in 96-well format. Preadipocytes and differentiated adipocytes are treated with the compound of interest. The activity of the reporter gene will be monitored by the fluorescent product of the Imagene (Molecular probes) β galactosidase substrate with a Cytofluor fluorescence plate reader.

The use of the rat β_3 promoter is not limited to <u>in vitro</u> analysis. The genetic

elements controlling fat-specific expression can be used to target the expression of transgenes to adipocytes of transgenic animals.

EXAMPLE 11

Isolation of the introns within the human and murine β_3 receptor gene.

Our discovery of an additional exon in the human and murine β_3 receptor genes allows for the cloning and elucidation of the correct genetic structure of the human and murine β_3 receptor genes. The genetic sequences that intervene the exons in the human gene are obtained by screening a commercially available human genomic library (Clontech) with a radiolabelled BamHI to XbaI fragment of p184 (Fig. 10B). The resulting clone is digested with BamHI, and the bands that hybridize to the full EcoRI to XbaI insert of p184 are gel-isolated and cloned into BamHI linearized, phosphatase-treated pGEM-7z. To obtain the sequences that intervene the novel mouse exons, a commercially-available mouse genomic library (Clontech) is screened with a radiolabelled probe derived from p158, which encodes the novel mouse exons described above.

Alternative methods include PCR using oligonucleotides that hybridize to p184 or p158.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Granneman, James G.
Lahners, Kristine N.
Rao, Donald D.

(ii) TITLE OF INVENTION: β3-ADRENERGIC RECEPTOR PROTEIN AND DNA ENCODING SAME

(iii) NUMBER OF SEQUENCES: 9

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: REISING, ETHINGTON, BARNARD, PERRY & MILTON
(B) STREET: 201 W. Big Beaver - Ste. 400; P.O. Box 4390
(C) CITY: Troy
(D) STATE: Michigan
(E) COUNTRY: USA
(F) ZIP: 48099

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
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(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Kohn, Kenneth I.
(B) REGISTRATION NUMBER: 30,955
(C) REFERENCE/DOCKET NUMBER: P-324(WSU)

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (313) 689-3554

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1227 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1224

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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 1               5                  10                  15
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Leu Pro Thr Leu Ala Pro Asn Thr Ala Asn Thr Ser Gly Leu Pro Gly
            20                  25                  30

GTT CCG TGG GAG GCG GCC CTA GCC GGG GCC CTG CTG GCG CTG GCG GTG         144
Val Pro Trp Glu Ala Ala Leu Ala Gly Ala Leu Leu Ala Leu Ala Val
        35                  40                  45

CTG GCC ACC GTG GGA GGC AAC CTG CTG GTC ATC GTG GCC ATC GCC TGG         192
Leu Ala Thr Val Gly Gly Asn Leu Leu Val Ile Val Ala Ile Ala Trp
    50                  55                  60

ACT CCG AGA CTC CAG ACC ATG ACC AAC GTG TTC GTG ACT TCG CTG GCC         240
Thr Pro Arg Leu Gln Thr Met Thr Asn Val Phe Val Thr Ser Leu Ala
65                  70                  75                  80

GCA GCC GAC CTG GTG ATG GGA CTC CTG GTG GTG CCG CCG GCG GCC ACC         288
Ala Ala Asp Leu Val Met Gly Leu Leu Val Val Pro Pro Ala Ala Thr
                85                  90                  95

TTG GCG CTG ACT GGC CAC TGG CCG TTG GGC GCC ACT GGC TGC GAG CTG         336
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            100                 105                 110

TGG ACC TCG GTG GAC GTG CTG TGT GTG ACC GCC AGC ATC GAA ACC CTG         384
Trp Thr Ser Val Asp Val Leu Cys Val Thr Ala Ser Ile Glu Thr Leu
        115                 120                 125

TGC GCC CTG GCC GTG GAC CGC TAC CTG GCT GTG ACC AAC CCG CTG CGT         432
Cys Ala Leu Ala Val Asp Arg Tyr Leu Ala Val Thr Asn Pro Leu Arg
    130                 135                 140

TAC GGC GCA CTG GTC ACC AAG CGC TGC GCC CGG ACA GCT GTG GTC CTG         480
Tyr Gly Ala Leu Val Thr Lys Arg Cys Ala Arg Thr Ala Val Val Leu
145                 150                 155                 160

GTG TGG GTC GTG TCG GCC GCG GTG TCG TTT GCG CCC ATC ATG AGC CAG         528
Val Trp Val Val Ser Ala Ala Val Ser Phe Ala Pro Ile Met Ser Gln
                165                 170                 175

TGG TGG CGC GTA GGG GCC GAC GCC GAG GCG CAG CGC TGC CAC TCC AAC         576
Trp Trp Arg Val Gly Ala Asp Ala Glu Ala Gln Arg Cys His Ser Asn
            180                 185                 190

CCG CGC TGC TGT GCC TTC GCC TCC AAC ATG CCC TAC GTG CTG CTG TCC         624
Pro Arg Cys Cys Ala Phe Ala Ser Asn Met Pro Tyr Val Leu Leu Ser
        195                 200                 205

TCC TCC GTC TCC TTC TAC CTT CCT CTT CTC GTG ATG CTC TTC GTC TAC         672
Ser Ser Val Ser Phe Tyr Leu Pro Leu Leu Val Met Leu Phe Val Tyr
    210                 215                 220

GCG CGG GTT TTC GTG GTG GCT ACG CGC CAG CTG CGC TTG CTG CGC GGG         720
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225                 230                 235                 240

GAG CTG GGC CGC TTT CCG CCC GAG GAG TCT CCG CCG GCG CCG TCG CGC         768
Glu Leu Gly Arg Phe Pro Pro Glu Glu Ser Pro Pro Ala Pro Ser Arg
                245                 250                 255

TCT CTG GCC CCG GCC CCG GTG GGG ACG TGC GCT CCG CCC GAA GGG GTG         816
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        275                 280                 285

CGG GCC CTG TGC ACC TTG GGT CTC ATC ATG GGC ACC TTC ACT CTC TGC        912
Arg Ala Leu Cys Thr Leu Gly Leu Ile Met Gly Thr Phe Thr Leu Cys
    290                 295                 300

TGG TTG CCC TTC TTT CTG GCC AAC GTG CTG CGC GCC CTG GGG GGC CCC        960
Trp Leu Pro Phe Phe Leu Ala Asn Val Leu Arg Ala Leu Gly Gly Pro
305                 310                 315                 320

TCT CTA GTC CCG GGC CCG GCT TTC CTT GCC CTG AAC TGG CTA GGT TAT       1008
Ser Leu Val Pro Gly Pro Ala Phe Leu Ala Leu Asn Trp Leu Gly Tyr
                325                 330                 335

GCC AAT TCT GCC TTC AAC CCG CTC ATC TAC TGC CGC AGC CCG GAC TTT       1056
Ala Asn Ser Ala Phe Asn Pro Leu Ile Tyr Cys Arg Ser Pro Asp Phe
            340                 345                 350

CGC AGC GCC TTC CGC CGT CTT CTG TGC CGC TGC GGC CGT CGC CTG CCT       1104
Arg Ser Ala Phe Arg Arg Leu Leu Cys Arg Cys Gly Arg Arg Leu Pro
        355                 360                 365

CCG GAG CCC TGC GCC GCC GCC CGC CCG GCC CTC TTC CCC TCG GGC GTT       1152
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    370                 375                 380

CCT GCG GCC CGG AGC AGC CCA GCG CAG CCC AGG CTT TGC CAA CGG CTC       1200
Pro Ala Ala Arg Ser Ser Pro Ala Gln Pro Arg Leu Cys Gln Arg Leu
385                 390                 395                 400

GAC GGG GCT TCT TGG GGA GTT TCT TAG                                   1227
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                405
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 408 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Val Pro Trp Glu Ala Ala Leu Ala Gly Ala Leu Leu Ala Leu Ala Val
        35                  40                  45

Leu Ala Thr Val Gly Gly Asn Leu Leu Val Ile Val Ala Ile Ala Trp
    50                  55                  60

Thr Pro Arg Leu Gln Thr Met Thr Asn Val Phe Val Thr Ser Leu Ala
65                  70                  75                  80
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Ala Ala Asp Leu Val Met Gly Leu Leu Val Val Pro Pro Ala Ala Thr
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Leu Ala Leu Thr Gly His Trp Pro Leu Gly Ala Thr Gly Cys Glu Leu
100 105 110

Trp Thr Ser Val Asp Val Leu Cys Val Thr Ala Ser Ile Glu Thr Leu
115 120 125

Cys Ala Leu Ala Val Asp Arg Tyr Leu Ala Val Thr Asn Pro Leu Arg
130 135 140

Tyr Gly Ala Leu Val Thr Lys Arg Cys Ala Arg Thr Ala Val Val Leu
145 150 155 160

Val Trp Val Val Ser Ala Ala Val Ser Phe Ala Pro Ile Met Ser Gln
165 170 175

Trp Trp Arg Val Gly Ala Asp Ala Glu Ala Gln Arg Cys His Ser Asn
180 185 190

Pro Arg Cys Cys Ala Phe Ala Ser Asn Met Pro Tyr Val Leu Leu Ser
195 200 205

Ser Ser Val Ser Phe Tyr Leu Pro Leu Leu Val Met Leu Phe Val Tyr
210 215 220

Ala Arg Val Phe Val Val Ala Thr Arg Gln Leu Arg Leu Leu Arg Gly
225 230 235 240

Glu Leu Gly Arg Phe Pro Pro Glu Glu Ser Pro Pro Ala Pro Ser Arg
245 250 255

Ser Leu Ala Pro Ala Pro Val Gly Thr Cys Ala Pro Pro Glu Gly Val
260 265 270

Pro Ala Cys Gly Arg Arg Pro Ala Arg Leu Leu Pro Leu Arg Glu His
275 280 285

Arg Ala Leu Cys Thr Leu Gly Leu Ile Met Gly Thr Phe Thr Leu Cys
290 295 300

Trp Leu Pro Phe Phe Leu Ala Asn Val Leu Arg Ala Leu Gly Gly Pro
305 310 315 320

Ser Leu Val Pro Gly Pro Ala Phe Leu Ala Leu Asn Trp Leu Gly Tyr
325 330 335

Ala Asn Ser Ala Phe Asn Pro Leu Ile Tyr Cys Arg Ser Pro Asp Phe
340 345 350

Arg Ser Ala Phe Arg Arg Leu Leu Cys Arg Cys Gly Arg Arg Leu Pro
355 360 365

Pro Glu Pro Cys Ala Ala Ala Arg Pro Ala Leu Phe Pro Ser Gly Val
370 375 380

Pro Ala Ala Arg Ser Ser Pro Ala Gln Pro Arg Leu Cys Gln Arg Leu
385 390 395 400

Asp Gly Ala Ser Trp Gly Val Ser
405

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 870 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(ix) FEATURE:
(A) NAME/KEY: exon
(B) LOCATION: 1..195

(ix) FEATURE:
(A) NAME/KEY: exon
(B) LOCATION: 196..870

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..215

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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 1               5                  10                  15

AGC GCC TTC CGC CGT CTT CTG TGC CGC TGC GGC CGT CGC CTG CCT CCG        96
Ser Ala Phe Arg Arg Leu Leu Cys Arg Cys Gly Arg Arg Leu Pro Pro
            20                  25                  30

GAG CCC TGC GCC GCC GCC CGC CCG GCC CTC TTC CCC TCG GGC GTT CCT       144
Glu Pro Cys Ala Ala Ala Arg Pro Ala Leu Phe Pro Ser Gly Val Pro
        35                  40                  45

GCG GCC CGG AGC AGC CCA GCG CAG CCC AGG CTT TGC CAA CGG CTC GAC       192
Ala Ala Arg Ser Ser Pro Ala Gln Pro Arg Leu Cys Gln Arg Leu Asp
    50                  55                  60

GGG GCT TCT TGG GGA GTT TCT TA GGCCTGAAGG ACAAGAAGCA ACAACTCTGT       245
Gly Ala Ser Trp Gly Val Ser
65                  70

TGATCAGAAC CTGTGGAAAA CCTCTGGCCT CTGTTCAGAA TGAGTCCCAT GGGATTCCCC     305

GGCTGTGACA CTCTACCCTC CAGAACCTGA CGACTGGGCC ATGTGACCCA AGGAGGGATC     365

CTTACCAAGT GGGTTTTCAC CATCCTCTTG CTCTCTGTCT GAGAGATGTT TTCTAAACCC     425

CAGCCTTGAA CTTCACTCCT CCCTCAGTGG TAGTGTCCAG GTGCCGTGGA GCAGCAGGCT     485

GGCTTTGGTA GGGGCACCCA TCACCCGGCT TGCCTGTGCA GTCAGTGAGT GCTTAGGGCA     545

AAGAGAGCTC CCCTGGTTCC ATTCCTTCTG CCACCCAAAC CCTGATGAGA CCTTAGTGTT     605

CTCCAGGCTC TGTGGCCCAG GCTGAGAGCA GCAGGGTAGA AAAGACCAAG ATTTGGGGTT     665

TTATCTCTGG TTCCCTTATT ACTGCTCTCA AGCAGTGGCC TCTCTCACTT TAGCCATGGA     725

ATGGCTCCGA TCTACCTCAC AGCAGTGTCA GAAGGACTTC GCCAGGGTTT TGGGAGCTCC     785
```

AGGGTTCATA AGAAGGTGAA CCATTAGAAC AGATCCCTTC TTTTCCTTTT GCAATCAGAT 845

AAATAAATAT CACTGAATGC AGTTC 870

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Asn Ser Ala Phe Asn Pro Leu Ile Tyr Cys Arg Ser Pro Asp Phe Arg
 1               5                  10                  15

Ser Ala Phe Arg Arg Leu Leu Cys Arg Cys Gly Arg Arg Leu Pro Pro
            20                  25                  30

Glu Pro Cys Ala Ala Ala Arg Pro Ala Leu Phe Pro Ser Gly Val Pro
        35                  40                  45

Ala Ala Arg Ser Ser Pro Ala Gln Pro Arg Leu Cys Gln Arg Leu Asp
    50                  55                  60

Gly Ala Ser Trp Gly Val Ser
65                  70
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2005 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 51..1250

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
TAAGCCAGCG GGTCTGGGGG GAAAACTTCC CATCCCAGAC GCGACACGAG ATG GCT      56
                                                          Met Ala
                                                            1

CCG TGG CCT CAC AAA AAC GGC TCT CTG GCT TTC TGG TCA GAC GCC CCC     104
Pro Trp Pro His Lys Asn Gly Ser Leu Ala Phe Trp Ser Asp Ala Pro
          5                  10                  15

ACC TTG GAC CCC AGT GCA GCC AAC ACC AGT GGG TTG CCA GGG GTG CCA     152
Thr Leu Asp Pro Ser Ala Ala Asn Thr Ser Gly Leu Pro Gly Val Pro
     20                  25                  30

TGG GCA GCG GCA TTG GCT GGA GCA TTG CTG GCG CTG GCC ACG GTG GGA     200
Trp Ala Ala Ala Leu Ala Gly Ala Leu Leu Ala Leu Ala Thr Val Gly
 35                  40                  45                  50
```

```
GGC AAC CTG CTG GTA ATC ACA GCT ATC GCC CGC ACG CCG AGA CTA CAG        248
Gly Asn Leu Leu Val Ile Thr Ala Ile Ala Arg Thr Pro Arg Leu Gln
                 55                  60                  65

ACC ATA ACC AAC GTG TTC GTG ACT TCG CTG GCC ACA GCT GAC TTG GTA        296
Thr Ile Thr Asn Val Phe Val Thr Ser Leu Ala Thr Ala Asp Leu Val
             70                  75                  80

GTG GGA CTC CTC GTA ATG CCA CCA GGG GCC ACA TTG GCG CTG ACT GGC        344
Val Gly Leu Leu Val Met Pro Pro Gly Ala Thr Leu Ala Leu Thr Gly
         85                  90                  95

CAC TGG CCC TTG GGC GCA ACT GGC TGC GAG CTG TGG ACG TCA GTG GAC        392
His Trp Pro Leu Gly Ala Thr Gly Cys Glu Leu Trp Thr Ser Val Asp
     100                 105                 110

GTG CTC TGT GTA ACT GCC AGC ATC GAG ACC CTG TGC GCC CTG GCT GTA        440
Val Leu Cys Val Thr Ala Ser Ile Glu Thr Leu Cys Ala Leu Ala Val
115                 120                 125                 130

GAC CGC TAC CTA GCC GTC ACC AAC CCT CTG CGT TAC GGC ACG CTG GTT        488
Asp Arg Tyr Leu Ala Val Thr Asn Pro Leu Arg Tyr Gly Thr Leu Val
                 135                 140                 145

ACC AAG CGC CGC GCC CGG GCG GCA GTA GTC CTG GTG TGG ATC GTG TCC        536
Thr Lys Arg Arg Ala Arg Ala Ala Val Val Leu Val Trp Ile Val Ser
             150                 155                 160

GCC ACC GTG TCC TTT GCG CCC ATC ATG AGC CAG TGG TGG CGT GTA GGG        584
Ala Thr Val Ser Phe Ala Pro Ile Met Ser Gln Trp Trp Arg Val Gly
         165                 170                 175

GCA GAC GCT GAG GCG CAA GAG TGT CAC TCC AAT CCG CGC TGC TGT TCC        632
Ala Asp Ala Glu Ala Gln Glu Cys His Ser Asn Pro Arg Cys Cys Ser
     180                 185                 190

TTT GCC TCC AAT ATG CCC TAC GCG CTG CTC TCC TCC TCC GTC TCC TTC        680
Phe Ala Ser Asn Met Pro Tyr Ala Leu Leu Ser Ser Ser Val Ser Phe
195                 200                 205                 210

TAC CTT CCC CTC CTT GTG ATG CTC TTC GTC TAT GCT CGA GTG TTC GTC        728
Tyr Leu Pro Leu Leu Val Met Leu Phe Val Tyr Ala Arg Val Phe Val
                 215                 220                 225

GTA GCT AAG CGC CAG CGG CGT TTG CTG CGC CGG GAG CTG GGC CGT TTT        776
Val Ala Lys Arg Gln Arg Arg Leu Leu Arg Arg Glu Leu Gly Arg Phe
             230                 235                 240

CCG CCC GAG GAG TCT CCG CGG TCT CCG TCG CGC TCT CCA TCC CCT GCC        824
Pro Pro Glu Glu Ser Pro Arg Ser Pro Ser Arg Ser Pro Ser Pro Ala
         245                 250                 255

ACA GTC GGG ACA CCC ACG GCA TCG GAT GGA GTG CCC TCC TGC GGG CGG        872
Thr Val Gly Thr Pro Thr Ala Ser Asp Gly Val Pro Ser Cys Gly Arg
     260                 265                 270

CGG CCT GCG CGC CTC CTA CCG CTC GGG GAA CAC CGC GCC CTG CGC ACC        920
Arg Pro Ala Arg Leu Leu Pro Leu Gly Glu His Arg Ala Leu Arg Thr
275                 280                 285                 290

TTG GGT CTC ATT ATG GGC ATC TTC TCT CTG TGC TGG CTG CCC TTC TTT        968
Leu Gly Leu Ile Met Gly Ile Phe Ser Leu Cys Trp Leu Pro Phe Phe
                 295                 300                 305
```

```
CTG GCC AAC GTG CTG CGC GCA CTC GTG GGG CCC TCC CTA GTT CCC AGC      1016
Leu Ala Asn Val Leu Arg Ala Leu Val Gly Pro Ser Leu Val Pro Ser
            310                 315                 320

GGA GTT TTC ATC GCC CTG AAC TGG TTG GGC TAT GCC AAC TCT GCC TTC      1064
Gly Val Phe Ile Ala Leu Asn Trp Leu Gly Tyr Ala Asn Ser Ala Phe
        325                 330                 335

AAC CCG CTC ATC TAC TGC CGC AGC CCG GAC TTT CGC GAC GCC TTC CGT      1112
Asn Pro Leu Ile Tyr Cys Arg Ser Pro Asp Phe Arg Asp Ala Phe Arg
    340                 345                 350

CGT CTT CTG TGC AGC TAC GGT GGC CGT GGA CCG GAA GAG CCA CGC GTG      1160
Arg Leu Leu Cys Ser Tyr Gly Gly Arg Gly Pro Glu Glu Pro Arg Val
355                 360                 365                 370

GTC ACC TTC CCA GCT AGC CCT GTT GCG TCC AGG CAG AAC TCA CCG CTC      1208
Val Thr Phe Pro Ala Ser Pro Val Ala Ser Arg Gln Asn Ser Pro Leu
                375                 380                 385

AAC AGG TTT GAT GGC TAT GAA GGT GAG CGT CCA TTT CCC ACA              1250
Asn Arg Phe Asp Gly Tyr Glu Gly Glu Arg Pro Phe Pro Thr
            390                 395                 400

TGAAGGACCA TGGAGATCTA GCAAGGAGCC TGACTTCTGG AGAAATTTTT TTTTAAGACA    1310

GAAAGACAAG CAACGTCCAT GGATGCAAAC CTTTTATCAG CCCTTGATTC TGCTCAGAGT    1370

GAGTTCCCAG GAACCGCAAC TCTCCAGACC ATGCATAGAC CACAGAATGT AAAGGGGAAA    1430

TCTTACCAAA TGGGTTTACC ATCTTCTCTC TCTTCGTGAG AGTGTCTATA GGCCACCTTG    1490

AACTTCGCTA CTACCTCAGC CGCCGGATAT CAGCCACCCT GCGTTGACTG CCTGGGAGGA    1550

GCTGCGTTCC CACCACCACC CTGCTTATTA TGTTTGTGCT GGATGCTTAG GGCTAAGAAA    1610

GCACCCTTAC CTACCTCCCT TCCTACGCTT TCCTGACCCC ATGAATGACT TTTGTCTCCA    1670

CAAATCACTC TGTCTCCAGG TTCTGTGTTC CCAGTCTCTG TGTCTCTGGT TAGTTGGAAA    1730

GCAGGAAACC CGGCGGGGGA GGCGGGGGAG GGGGGGAACG ACCAAGTTTG AGGTTTTGTC    1790

CCTGGCTCCT CACTACAGCT CTCTAAACAT CATCTTGGAC CATCTCTCAC AATAGGCACA    1850

AAACAGCTCT AATCTACCTC ACTCTTAGGA CTTCAAGGTT TGGGAGAAAT TCCAGGGTTC    1910

CTGGGAAGAA GTCAAACCAT TGGAATGGGT CCCTTTTGGC GTTAAAATCA AATTAATAAA    1970

TATTATTGAA TGTGAAAAAA AAAAAAAAAT CTAGA                               2005
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 400 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Ala Pro Trp Pro His Lys Asn Gly Ser Leu Ala Phe Trp Ser Asp
 1               5                  10                  15
```

```
Ala Pro Thr Leu Asp Pro Ser Ala Ala Asn Thr Ser Gly Leu Pro Gly
            20                  25                  30

Val Pro Trp Ala Ala Ala Leu Ala Gly Ala Leu Leu Ala Leu Ala Thr
        35                  40                  45

Val Gly Gly Asn Leu Leu Val Ile Thr Ala Ile Ala Arg Thr Pro Arg
    50                  55                  60

Leu Gln Thr Ile Thr Asn Val Phe Val Thr Ser Leu Ala Thr Ala Asp
65                  70                  75                  80

Leu Val Val Gly Leu Leu Val Met Pro Pro Gly Ala Thr Leu Ala Leu
                85                  90                  95

Thr Gly His Trp Pro Leu Gly Ala Thr Gly Cys Glu Leu Trp Thr Ser
            100                 105                 110

Val Asp Val Leu Cys Val Thr Ala Ser Ile Glu Thr Leu Cys Ala Leu
        115                 120                 125

Ala Val Asp Arg Tyr Leu Ala Val Thr Asn Pro Leu Arg Tyr Gly Thr
    130                 135                 140

Leu Val Thr Lys Arg Arg Ala Arg Ala Ala Val Val Leu Val Trp Ile
145                 150                 155                 160

Val Ser Ala Thr Val Ser Phe Ala Pro Ile Met Ser Gln Trp Trp Arg
                165                 170                 175

Val Gly Ala Asp Ala Glu Ala Gln Glu Cys His Ser Asn Pro Arg Cys
            180                 185                 190

Cys Ser Phe Ala Ser Asn Met Pro Tyr Ala Leu Leu Ser Ser Ser Val
        195                 200                 205

Ser Phe Tyr Leu Pro Leu Leu Val Met Leu Phe Val Tyr Ala Arg Val
    210                 215                 220

Phe Val Val Ala Lys Arg Gln Arg Arg Leu Leu Arg Arg Glu Leu Gly
225                 230                 235                 240

Arg Phe Pro Pro Glu Glu Ser Pro Arg Ser Pro Ser Arg Ser Pro Ser
                245                 250                 255

Pro Ala Thr Val Gly Thr Pro Thr Ala Ser Asp Gly Val Pro Ser Cys
            260                 265                 270

Gly Arg Arg Pro Ala Arg Leu Leu Pro Leu Gly Glu His Arg Ala Leu
        275                 280                 285

Arg Thr Leu Gly Leu Ile Met Gly Ile Phe Ser Leu Cys Trp Leu Pro
    290                 295                 300

Phe Phe Leu Ala Asn Val Leu Arg Ala Leu Val Gly Pro Ser Leu Val
305                 310                 315                 320

Pro Ser Gly Val Phe Ile Ala Leu Asn Trp Leu Gly Tyr Ala Asn Ser
                325                 330                 335

Ala Phe Asn Pro Leu Ile Tyr Cys Arg Ser Pro Asp Phe Arg Asp Ala
            340                 345                 350
```

Phe Arg Arg Leu Leu Cys Ser Tyr Gly Gly Arg Gly Pro Glu Glu Pro
355 360 365

Arg Val Val Thr Phe Pro Ala Ser Pro Val Ala Ser Arg Gln Asn Ser
370 375 380

Pro Leu Asn Arg Phe Asp Gly Tyr Glu Gly Glu Arg Pro Phe Pro Thr
385 390 395 400

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 687 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: intron
(B) LOCATION: 9..402

(ix) FEATURE:
(A) NAME/KEY: exon
(B) LOCATION: 403..470

(ix) FEATURE:
(A) NAME/KEY: intron
(B) LOCATION: 471..674

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CTCAACAGGT AGGCGACGCA GGCAGAGGAC TGGAGTCTGG GTGGGGACGC CTCTGTCTCT 60

ATTTTTGAGT TTGAGGGTTG GGGGAGGAGA AGGTGTAGAC AGGGCTTTTG TCTCGAGAGG 120

ACAGAAAAGG AGTAAGAACA GAATCGGGAT CTAGGGCCCT TCCTTTTATT GGATCCAATC 180

CCTGGGTCTG AGGCAAAGGA GGAAAGGGAA ATTTGTTCAC CTTGGGACCA GGTGAGCCCC 240

ACAGGTTTCT GCCAGCAGGT TTCTGACCTC TCTGGTTGCC TCTAGTTTGG ATCTTTTTAG 300

TTCTATTCTC CAGGCGCCCA GGTATCACTA ACTTGTCTGG GACATCCATA GACAGCAATG 360

GACATGTCAA GTCCTCTGCC TCAGTTCCGC TTTCTTTCAA AGGTTTGATG GCTATGAAGG 420

TGAGCGTCCA TTTCCCACAT GAAGGACCAT GGAGATCTAG CAAGGAGCCT GTGAGTTGAA 480

TTTGAGCTGC TTTTCTCCCT CAGGGACTGG ATTCGAGGTG TAGGGTGGGA TGAGGGAGGG 540

TGCAGGATGA TCCCTATATC TTTGAAAAGT AAATATGCTA TTCAGGGTTC CTGAGTCACT 600

CCCCTCTTAC CTCCAGTGCT TGATCCGCAC CTCCTTGACT GGTTACCCCA AGAAATATTG 660

TTTCCGTTTT GCAGGACTTC TGGAGAA 687

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 176 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(ix) FEATURE:
(A) NAME/KEY: exon
(B) LOCATION: 1..60

(ix) FEATURE:
(A) NAME/KEY: exon
(B) LOCATION: 61..176

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..97

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
A CGC GCA GTC ACC TTC CCA GCC AGC CCT GTT GAA GCC AGG CAG AGT          46
  Arg Ala Val Thr Phe Pro Ala Ser Pro Val Glu Ala Arg Gln Ser
    1               5                  10                  15

CCA CCG CTC AAC AGG TTT GAT GGC TAT GAA GGT GCG CGT CCG TTT CCC        94
Pro Pro Leu Asn Arg Phe Asp Gly Tyr Glu Gly Ala Arg Pro Phe Pro
                20                  25                  30

ACG TGAAGGGCCG TGAAGATCCA GCAAGGAAGC TGACTTCTGG GGATTTTTTT           147
Thr

TTTCCTCCAG AAAGACAAGC AACGTCCAT                                      176
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
Arg Ala Val Thr Phe Pro Ala Ser Pro Val Glu Ala Arg Gln Ser Pro
 1               5                  10                  15

Pro Leu Asn Arg Phe Asp Gly Tyr Glu Gly Ala Arg Pro Phe Pro Thr
            20                  25                  30
```

CLAIMS

1. A DNA sequence which encodes a mammalian β_3-adrenergic receptor in substantially pure form, functional equivalents thereof or a nucleic acid sequence which hybridizes thereto.

2. The DNA sequence of claim 1 which encodes human β_3-adrenergic receptor.

3. The DNA sequence of claim 2 which comprises genomic DNA in a combination with cDNA.

4. The gene of claim 2 which comprises the coding sequence depicted in Fig. 1 hereof.

5. A nucleic acid sequence of claim 1 which is cDNA.

6. A nucleic acid sequence of claim 5 which is p184 depicted in Fig. 10B hereof.

7. A nucleic acid sequence of claim 5 which is p192 described herein.

8. A nucleic acid sequence of claim 1 which is mRNA or cRNA.

9. The gene of claim 1 which encodes rodent β_3-adrenergic receptor.

10. The gene of claim 9 which encodes rat β_3-adrenergic receptor.

11. An oligonucleotide which hybridizes to the DNA sequence of claim 1.

12. An oligonucleotide which hybridizes to a nucleic acid of claim 1.

13. An oligonucleotide of claim 11 which carries a detectable label.

14. A vector which comprises the DNA of claim 1.

15. A host cell transformed with the vector of claim 14.

16. A vector which comprises the DNA of claim 2.

17. A host cell which is transformed with the vector of claim 16.

18. A host cell of claim 17 which does not express other β adrenergic receptors.

19. A vector which comprises the DNA of claim 4.

20. A vector of claim 19 which is a shuttle vector.

21. A host cell which is transformed with the vector of claim 20.

22. A host cell of claim 21 which does not express other β-adrenergic receptors

23. A method of preparing a β_3-adrenergic receptor which comprises culturing a host cell of claim 18.

24. A method of preparing a β_3-adrenergic receptor which comprises culturing a host cell of claim 22.

25. A method for monitoring the presence of human β_3 receptor gene which comprises extracting the mRNA from human adipose tissue and bringing said mRNA into contact with a nucleic acid sequence which hybridizes to the DNA sequence of claim 2.

26. The method of claim 25 which utilizes the nucleic acid sequence of claim 7.

27. The method of claim 26 wherein the mRNA hybridizes to the nucleic acid sequence of claim 6.

28. The method of claim 26 wherein the mRNA hybridizes to the nucleic acid sequence of claim 9.

29. A method for identifying a compound which affect the activity of the β_3-adrenergic receptor which comprises bringing said compound in contact with a host cell transformed

with a vector of claim 14 and detecting any change in the level of activity of the β_3-adrenergic receptor.

30. The method of claim 29 wherein the host cell is transformed with the vector of claim 16.

31. The method of claim 30 wherein the host cell does not express other β-adrenergic receptors.

32. The method of claim 29 herein the host cell is transformed with the vector of claim 19.

33. The method claim 32 wherein the host cell does not express other β-adrenergic receptors.

34. A β_3-adrenergic receptor protein in substantially pure form having the amino acid sequence depicted in Fig. 1 and functional equivalents thereof.

35. A β_3-adrenergic receptor protein produced by the expression of the DNA of claim 1.

36. The receptor protein of claim 35 which is human β_3-adrenergic receptor protein.

37. The receptor protein of claim 36 which is rodent β_3-adrenergic receptor protein.

38. A DNA sequence which encodes the first exon of β_3-adrenergic receptor wherein the nucleotide at position 1206 is changed from guanine (G) to a nucleotide selected from thymine (T), adenine (A) or cytosine (C).

39. The DNA sequence of claim 38 which encodes the first exon of human β_3-adrenergic receptor.

40. An antibody which is specific to the protein of claim 34.

41. The antibody of claim 40 which is a monoclonal antibody.

42. An antibody which is specific to the protein of claim 36.

43. The antibody of claim 42 which is a monoclonal antibody.

44. A DNA construct which comprises the fat-specific promoter and enhancer elements of the rodent β_3-adrenergic receptor.

45. The construct of claim 44 wherein the DNA sequence is selected from the introns and the 5'-flanking region.

46. The construct of claim 45 which is a vector.

47. The construct of claim 46 which contains a reporter gene whose transcription is modulated by the fat-specific promoter and enhancer elements of the rat β_3 receptor gene.

48. A host cell transfected with the construct of claim 47.

49. The host cell of claim 48 wherein the fat-specific DNA sequences are from the 5'-flanking region of the rat β_3 receptor gene.

50. The host cell of claim 48 wherein the fat-specific DNA sequences are from the introns of the rat β_3 receptor gene.

```
          10          20          30          40
          |           |           |           |
ATG GCT CCG TGG CCT CAC GAG AAC AGC TCT CTT GCC CCA TGG CCG
MET Ala Pro Trp Pro His Glu Asn Ser Ser Leu Ala Pro Trp Pro

    50          60          70          80          90
    |           |           |           |           |
GAC CTC CCC ACC CTG GCG CCC AAT ACC GCC AAC ACC AGT GGG CTG
Asp Leu Pro Thr Leu Ala Pro Asn Thr Ala Asn Thr Ser Gly Leu

         100         110         120         130
          |           |           |           |
CCA GGG GTT CCG TGG GAG GCG GCC CTA GCC GGG GCC CTG CTG GCG
Pro Gly Val Pro Trp Glu Ala Ala Leu Ala Gly Ala Leu Leu Ala

   140         150         160         170         180
    |           |           |           |           |
CTG GCG GTG CTG GCC ACC GTG GGA GGC AAC CTG CTG GTC ATC GTG
Leu Ala Val Leu Ala Thr Val Gly Gly Asn Leu Leu Val Ile Val

         190         200         210         220
          |           |           |           |
GCC ATC GCC TGG ACT CCG AGA CTC CAG ACC ATG ACC AAC GTG TTC
Ala Ile Ala Trp Thr Pro Arg Leu Gln Thr MET Thr Asn Val Phe

   230         240         250         260         270
    |           |           |           |           |
GTG ACT TCG CTG GCC GCA GCC GAC CTG GTG ATG GGA CTC CTG GTG
Val Thr Ser Leu Ala Ala Ala Asp Leu Val MET Gly Leu Leu Val

         280         290         300         310
          |           |           |           |
GTG CCG CCG GCG GCC ACC TTG GCG CTG ACT GGC CAC TGG CCG TTG
Val Pro Pro Ala Ala Thr Leu Ala Leu Thr Gly His Trp Pro Leu

   320         330         340         350         360
    |           |           |           |           |
GGC GCC ACT GGC TGC GAG CTG TGG ACC TCG GTG GAC GTG CTG TGT
Gly Ala Thr Gly Cys Glu Leu Trp Thr Ser Val Asp Val Leu Cys
```

Fig-1A

```
          370         380         390         400
GTG ACC GCC AGC ATC GAA ACC CTG TGC GCC CTG GCC GTG GAC CGC
Val Thr Ala Ser Ile Glu Thr Leu Cys Ala Leu Ala Val Asp Arg

   410         420         430         440         450
TAC CTG GCT GTG ACC AAC CCG CTG CGT TAC GGC GCA CTG GTC ACC
Tyr Leu Ala Val Thr Asn Pro Leu Arg Tyr Gly Ala Leu Val Thr

          460         470         480         490
AAG CGC TGC GCC CGG ACA GCT GTG GTC CTG GTG TGG GTC GTG TCG
Lys Arg Cys Ala Arg Thr Ala Val Val Leu Val Trp Val Val Ser

   500         510         520         530         540
GCC GCG GTG TCG TTT GCG CCC ATC ATG AGC CAG TGG TGG CGC GTA
Ala Ala Val Ser Phe Ala Pro Ile MET Ser Gln Trp Trp Arg Val

          550         560         570         580
GGG GCC GAC GCC GAG GCG CAG CGC TGC CAC TCC AAC CCG CGC TGC
Gly Ala Asp Ala Glu Ala Gln Arg Cys His Ser Asn Pro Arg Cys

   590         600         610         620         630
TGT GCC TTC GCC TCC AAC ATG CCC TAC GTG CTG CTG TCC TCC TCC
Cys Ala Phe Ala Ser Asn MET Pro Tyr Val Leu Leu Ser Ser Ser

          640         650         660         670
GTC TCC TTC TAC CTT CCT CTT CTC GTG ATG CTC TTC GTC TAC GCG
Val Ser Phe Tyr Leu Pro Leu Leu Val MET Leu Phe Val Tyr Ala

   680         690         700         710         720
CGG GTT TTC GTG GTG GCT ACG CGC CAG CTG CGC TTG CTG CGC GGG
Arg Val Phe Val Val Ala Thr Arg Gln Leu Arg Leu Leu Arg Gly

          730         740         750         760
GAG CTG GGC CGC TTT CCG CCC GAG GAG TCT CCG CCG GCG CCG TCG
Glu Leu Gly Arg Phe Pro Pro Glu Glu Ser Pro Pro Ala Pro Ser
```

Fig-1B

```
   770         780         790         800         810
CGC TCT CTG GCC CCG GCC CCG GTG GGG ACG TGC GCT CCG CCC GAA
Arg Ser Leu Ala Pro Ala Pro Val Gly Thr Cys Ala Pro Pro Glu

          820         830         840         850
GGG GTG CCC GCC TGC GGC CGG CGG CCC GCG CGC CTC CTG CCT CTC
Gly Val Pro Ala Cys Gly Arg Arg Pro Ala Arg Leu Leu Pro Leu

   860         870         880         890         900
CGG GAA CAC CGG GCC CTG TGC ACC TTG GGT CTC ATC ATG GGC ACC
Arg Glu His Arg Ala Leu Cys Thr Leu Gly Leu Ile MET Gly Thr

          910         920         930         940
TTC ACT CTC TGC TGG TTG CCC TTC TTT CTG GCC AAC GTG CTG CGC
Phe Thr Leu Cys Trp Leu Pro Phe Phe Leu Ala Asn Val Leu Arg

   950         960         970         980         990
GCC CTG GGG GGC CCC TCT CTA GTC CCG GGC CCG GCT TTC CTT GCC
Ala Leu Gly Gly Pro Ser Leu Val Pro Gly Pro Ala Phe Leu Ala

          1000        1010        1020        1030
CTG AAC TGG CTA GGT TAT GCC AAT TCT GCC TTC AAC CCG CTC ATC
Leu Asn Trp Leu Gly Tyr Ala Asn Ser Ala Phe Asn Pro Leu Ile

  1040        1050        1060        1070        1080
TAC TGC CGC AGC CCG GAC TTT CGC AGC GCC TTC CGC CGT CTT CTG
Tyr Cys Arg Ser Pro Asp Phe Arg Ser Ala Phe Arg Arg Leu Leu

          1090        1100        1110        1120
TGC CGC TGC GGC CGT CGC CTG CCT CCG GAG CCC TGC GCC GCC GCC
Cys Arg Cys Gly Arg Arg Leu Pro Pro Glu Pro Cys Ala Ala Ala

  1130        1140        1150        1160        1170
CGC CCG GCC CTC TTC CCC TCG GGC GTT CCT GCG GCC CGG AGC AGC
Arg Pro Ala Leu Phe Pro Ser Gly Val Pro Ala Ala Arg Ser Ser
```

Fig-1C

```
          1180          1190          1200          1210
           |             |             |             |
CCA GCG CAG CCC AGG CTT TGC CAA CGG CTC GAC GGG GCT TCT TGG
Pro Ala Gln Pro Arg Leu Cys Gln Arg Leu Asp Gly Ala Ser Trp

  1220
    |
GGA GTT TCT TAG
Gly Val Ser ---
```

Fig-1D

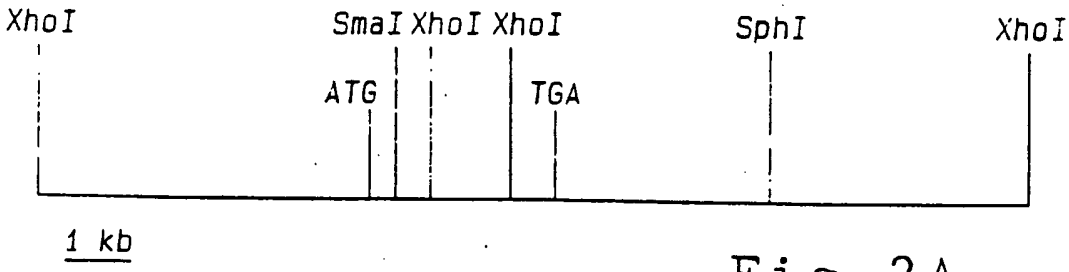

Fig-2A

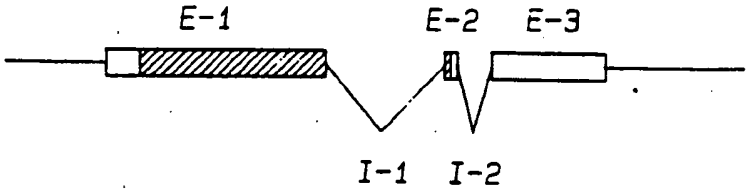

Fig-2B

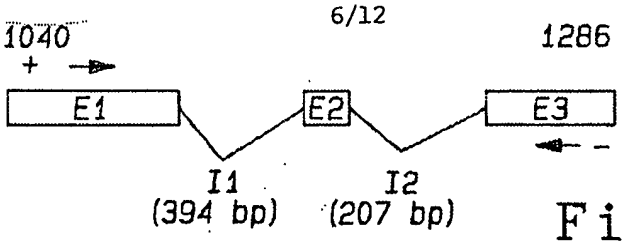

Fig-3A

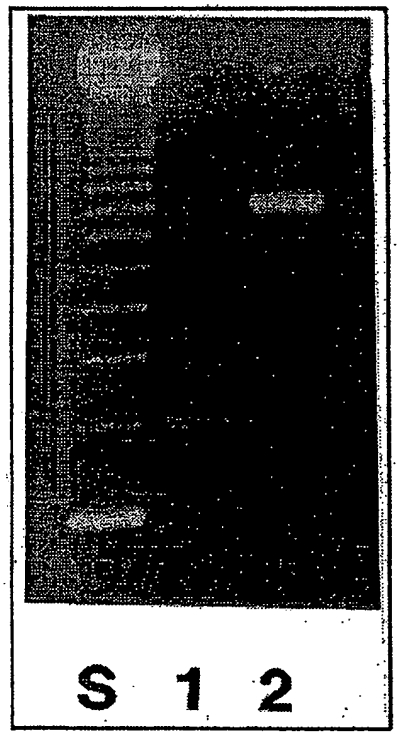

Fig-3B

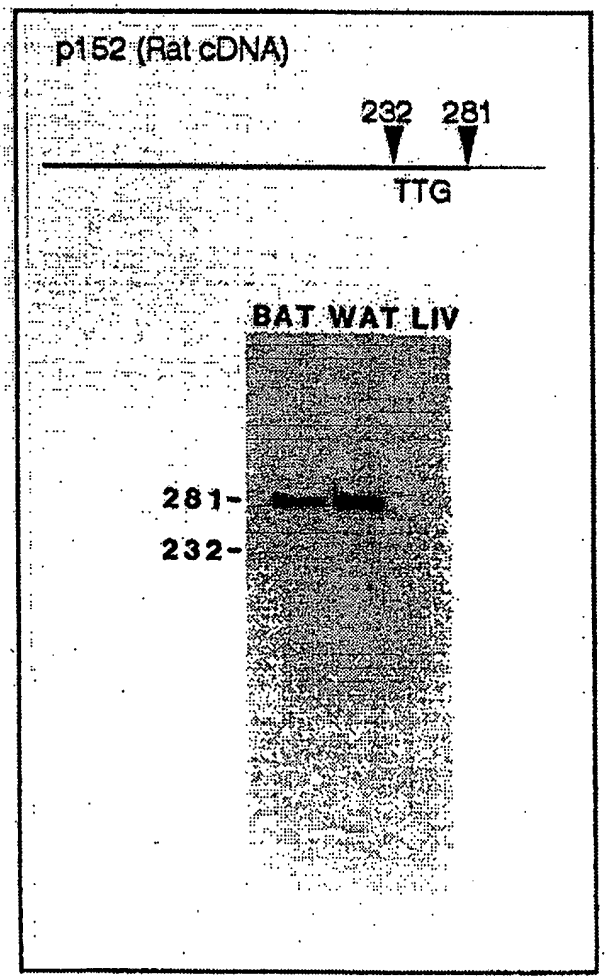

Fig-4

	GENOMIC	cDNA
Rat	CCG CTC AAC AGG TAG Pro Leu Asn Arg	CCG CTC AAC AGG TTT GAT GGC TAT GAA Pro Leu Asn Arg Phe Asp Gly Tyr Glu GGT GAG CGT CCA TTT CCC ACA TGA Gly Glu Arg Pro Phe Pro Thr STOP
Mouse	CCG CTC AAC AGG TAG Pro Leu Asn Arg	
Human	CGG CTC GAC GGG TAG Arg Leu Asp Gly	

Fig-5

...GTT GAA GCC AGG CAG AGT CCA CCG CTC AAC AGG TTT GAT GGC TAT
Val Glu Ala Arg Gln Ser Pro Pro Leu Asp Arg Phe Asp Gly Tyr

GAA GGT GCG CGT CCG TTT CCC ACG TGA AGGGCCGTGAAGATCCAGCAAG
Glu Gly Ala Arg Pro Phe Pro Thr ---

GAAGCTGACTTCTGGGGATTTTTTTTTTCCTCCAGAAAGACAAGCAACGTCCAT...

Fig-6

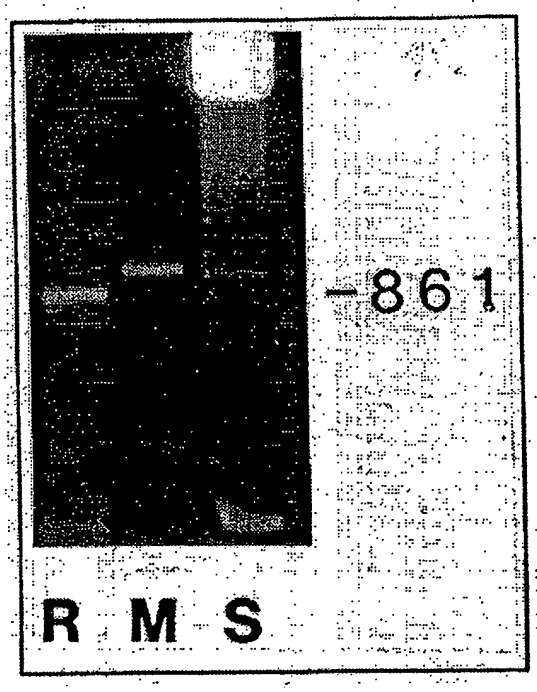

Fig-7

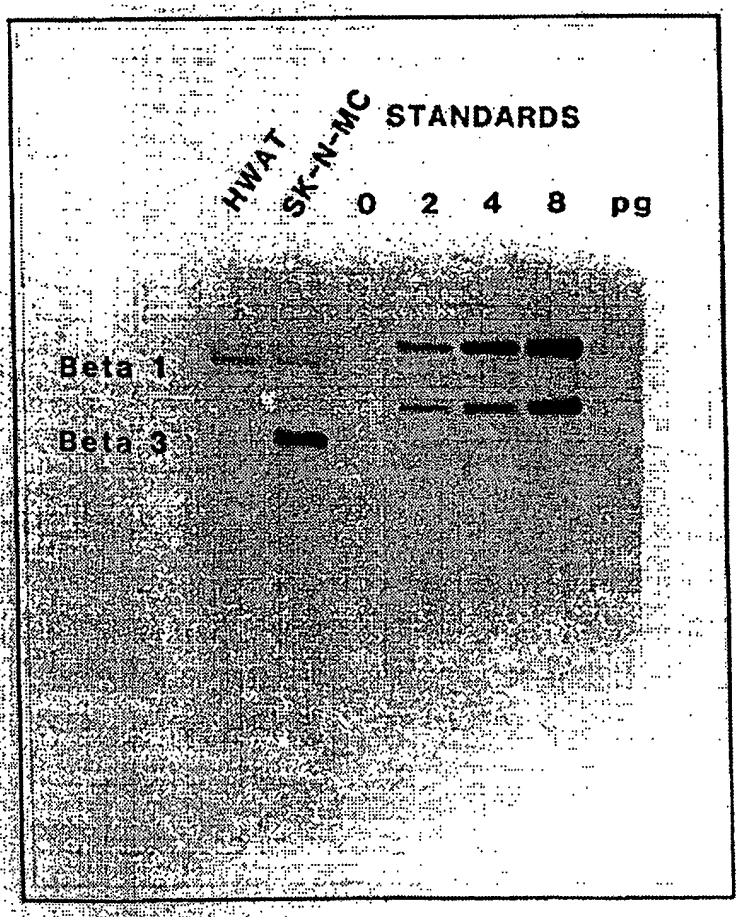

Fig-8

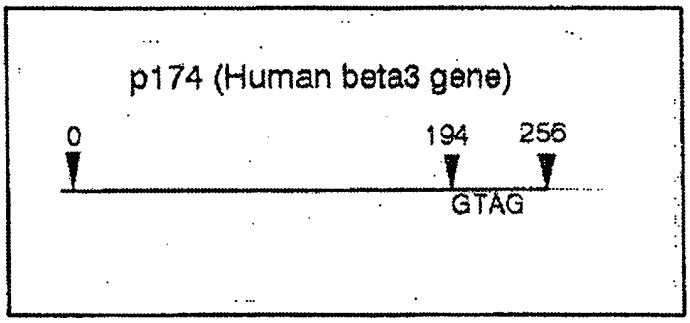
p174 (Human beta3 gene)
0
194
256
GTAG

Fig-9A

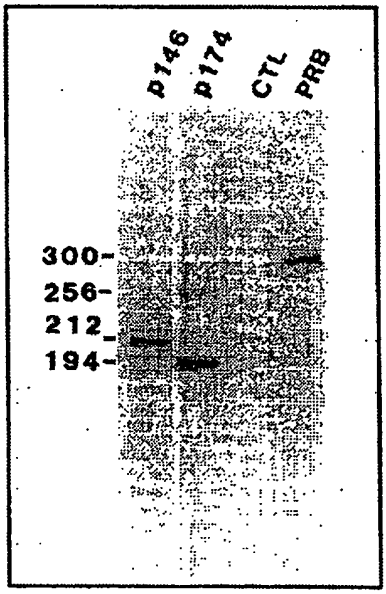
p146
p174
CTL
PRB
300-
256-
212-
194-

Fig-9B

(162 bp to begining of clone)... GCG CAG CCC AGG CTT TGC CAA
Ala Gln Pro Arg Leu Cys Gln

CGG CTC GAC GGG GCT TCT TGG GGA GTT TCT TAG GCCTGAAGGACAAGAA
Arg Leu Asp Gly Ala Ser Trp Gly Val Ser ---

GCAACAACTCTGTTGATCAGAACCTGTGGAAA...(680 bp to poly A)

Fig-10A

AATTCTGCCT	TCAACCCGCT	CATCTACTGC	CGCAGCCCGG	ACTTTCGCAG
CGCCTTCCGC	CGTCTTCTGT	GCCGCTGCGG	CCGTCGCCTG	CCTCCGGAGC
CCTGCGCCGC	CGCCCGCCCG	GCCCTCTTCC	CCTCGGGCGT	TCCTGCGGCC
CGGAGCAGCC	CAGCGCAGCC	CAGGCTTTGC	CAACGGCTCG	ACGGGGCTTC
TTGGGGAGTT	TCTTAGGCCT	GAAGGACAAG	AAGCAACAAC	TCTGTTGATC
AGAACCTGTG	GAAAACCTCT	GGCCTCTGTT	CAGAATGAGT	CCCATGGGAT
TCCCCGGCTG	TGACACTCTA	CCCTCCAGAA	CCTGACGACT	GGGCCATGTG
ACCCAAGGAG	GGATCCTTAC	CAAGTGGGTT	TTCACCATCC	TCTTGCTCTC
TGTCTGAGAG	ATGTTTTCTA	AACCCCAGCC	TTGAACTTCA	CTCCTCCCTC
AGTGGTAGTG	TCCAGGTGCC	GTGGAGCAGC	AGGCTGGCTT	TGGTAGGGGC
ACCCATCACC	CGGCTTGCCT	GTGCAGTCAG	TGAGTGCTTA	GGGCAAAGAG
AGCTCCCCTG	GTTCCATTCC	TTCTGCCACC	CAAACCCTGA	TGAGACCTTA
GTGTTCTCCA	GGCTCTGTGG	CCCAGGCTGA	GAGCAGCAGG	GTAGAAAAGA
CCAAGATTTG	GGGTTTTATC	TCTGGTTCCC	TTATTACTGC	TCTCAAGCAG
TGGCCTCTCT	CACTTTAGCC	ATGGAATGGC	TCCGATCTAC	CTCACAGCAG
TGTCAGAAGG	ACTTCGCCAG	GGTTTTGGGA	GCTCCAGGGT	TCATAAGAAG
GTGAACCATT	AGAACAGATC	CCTTCTTTTC	CTTTTGCAAT	CAGATAAATA
AATATCACTG	AATGCAGTTC			

Fig-10B

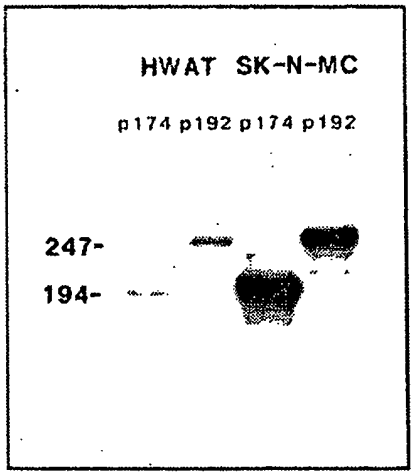

Fig-11

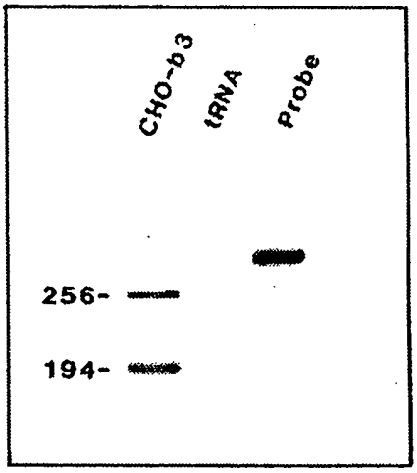

Fig-12

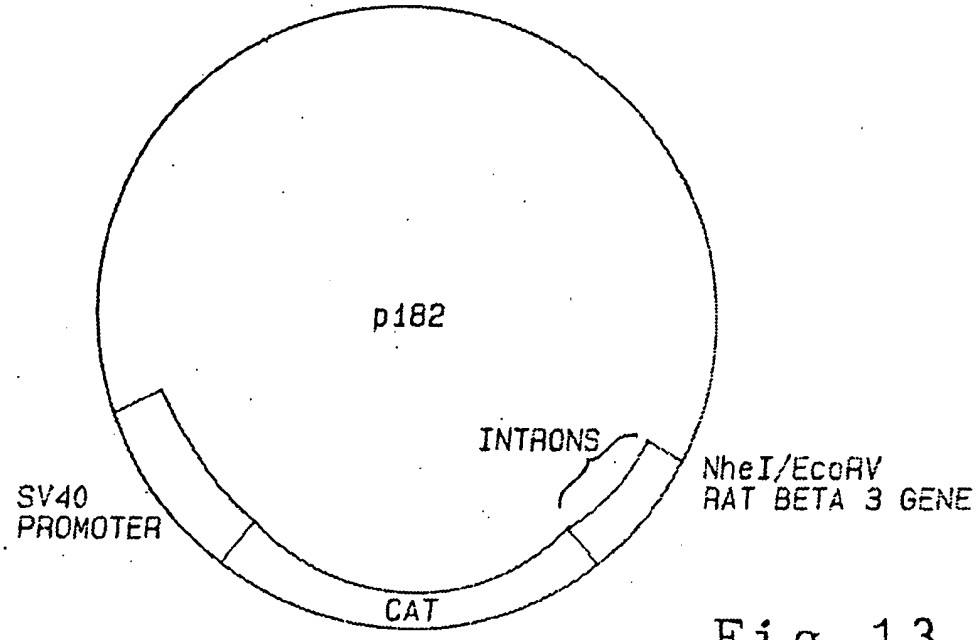

Fig-13

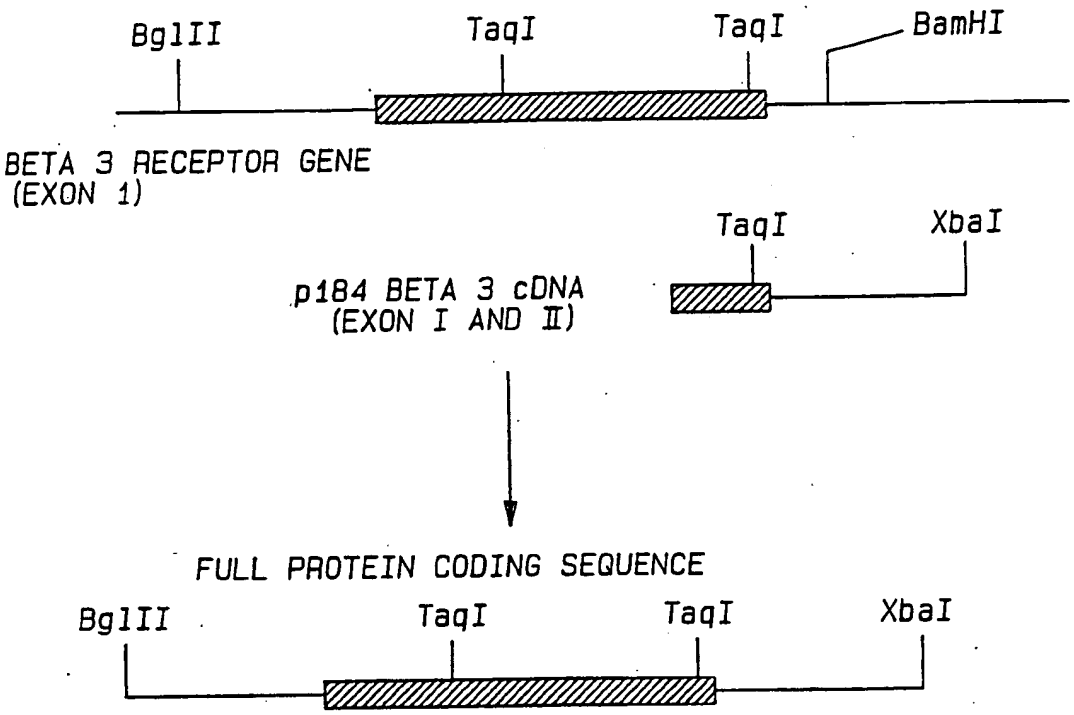

Fig-14

INTERNATIONAL SEARCH REPORT

tional application No.
PCT/US93/06733

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C12N 1/21, 15/00; C07K 13/00, 15/28
US CL :536/23.1; 530/350, 388.1; 435/69.1, 240.1, 320.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.1; 530/350, 388.1; 435/69.1, 240.1, 320.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	WO, A, 90/08775 (Emorine, et al) 09 August 1990, see abstract.	1-3, 5, 8-18, 23, 25, 27-31, 34, 35, 36, 40-43 37, 44-50; 4, 6, 7, 19-22, 24, 26, 32, 33, 38, 39

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
23 SEPTEMBER 1993	OCT 06 1993
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer ROBERT HILL Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/06733

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	Science, Volume 245, issued 08 September 1989, L. J. Emorine et al, "Molecular Characterization of the Human B3-Adrenergic Receptor", pages 1118-1121, especially figure 1.	1-3, 5, 8-18, 23, 25, 27-31, 34, 35, 36, 40-43 37, 44-50, 4, 6, 7, 19-22, 24, 26, 32, 33, 38, 39
X Y	Molecular Pharmacology, Volume 40, issued 1991, J. G. Granneman et al, "Molecular Cloning and Expression of the Rat B3-Adrenergic Receptor", pages 895-899, especially figures 1 and 2.	1-3, 5, 8-18, 23, 25, 27-31, 34-36, 37, 40-43, 44-50 4, 6, 7, 19-22, 24, 26, 32, 33, 38, 39
X Y	The EMBO Journal, Volume 10, No. 12, issued 1991, C. Nahmias et al, "Molecular Characterization of the Mouse B3-Adrenergic Receptor: Relationship with the Atypical Receptor of Adipocytes", pages 3721-3727, especially figure 1.	1-3, 5, 8-18, 23, 25, 27-31, 34-36, 37, 40-43, 44-50 4, 6,7, 19-22, 24, 26, 32, 33, 38, 39

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, Medline, Dialog
search terms: B3-adrenergic receptor, cloning, cDNA, adipocyte receptor

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